STUDIES ON TICK PYAEMIA WITH SPECIAL REFERENCE TO TRANSMISSION AND IMMUNITY.

Submitted as a Thesis for the Degree of Ph.D.
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by

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CONTENTS.

INTRODUCTION.

An enzootic staphylococcal pyaemia of young lambs has been recognised for fifty years as a disease of sheep stocks on tick infested pastures in Great Britain.

The disease was first described by McFadyean (1894) who differentiated it from louping-ill. Stewart and Ponsford (1937), in a survey of the incidence of various disease conditions in hill sheep in Northumberland, found that tick pyaemia was present in 50 of 110 dead lambs examined by them. Taylor, Holman and Gordon (1941) recorded tick pyaemia in Scotland and described attempts to transmit the disease artificially, by subcutaneous injection of Staphylococcus aureus, in lambs which had been infected with louping-ill and tick-borne fever. Tick pyaemia has also been recorded from Wales (Walton 1927, Rowlands 1945) and from Northern Ireland (Foggie 1943).

McDiarmid (1946 a.) describes a septicaemic form of the disease. For this reason he suggests that 'tick pyaemia' is a misnomer and uses the term 'enzootic staphylococcal infection'.

In the flocks under observation during the present study, such septicaemic cases were rare. The disease was a typical pyaemia with abscess formation in various parts of the body. The joints were most commonly involved. The more descriptive name 'tick pyaemia' /

pyaemia' is therefore retained.

The thesis is divided into three sections as follows:-

Part I is a study of the incidence of tick pyaemia in two tick-infested districts.

Part II is a description of experiments designed to show the source and manner of infection in the disease.

Part III describes attempts to produce immunity to the disease in young lambs.

PART I.

ON THE INCIDENCE OF TICK PYAEMIA OF LAMBS.

As there is no authentic record of enzootic staphylococcal pyaemia occurring in lambs on tick-free pastures, the disease has been associated with infestation by the common sheep tick, Ixodes ricinus, by all writers on the subject. McEwen (personal communication) has encountered the disease in lambs on pastures in Kent in which an infestation of ticks, which were probably of the genus Haemaphysalis, occurred.

In order to clarify the connection between tick infestation and the occurrence of the disease, a study of the incidence of tick pyaemia and of related factors was carried out in two districts where the disease is prevalent.

The Knowe, Kirkconnel, is situated in Nithsdale, North-west Dumfriesshire. This farm includes a dairy, and arable land, as well as the hill-sheep stock which grazes the uplands. The upland pasture carries a flock of 550 Cheviot ewes.

The area on the Stirlingshire shore of Loch Lomond between Ben Lomond and Balmaha is divided into four hirsels belonging to the Rowardennan estate, and a two-shepherd hirsel at Old Manse. The area is typical mountain pasture and carries a stock of 2,500 Black-face ewes. Sallochy hirsel, which lies in the middle of the group, is reputed to be the most heavily tick-infested.

Observations /

Observations were made on the following points:-

(a) The age and sex of the affected animals, and the seasonal incidence of tick pyaemia.

(b) The degree of tick-infestation.

(c) Ewes appear to have a complete age immunity to this pyaemia, but since it occurs in lambs, it is likely that ewes are exposed to a similar degree of infection. A study of the seasonal variations of the staphylococcal anti-haemolysins in the sera of ewes in the two tick-infested districts and of ewes on a tick-free farm was made.

(a) <u>The age and sex of the affected lambs, and the seasonal incidence of tick pyaemia</u>.

As it was not possible to examine every case of pyaemia reported, the total figures in this section are based on the shepherds' counts of lambs showing lameness or superficial abscesses. Post-mortem examination of a considerable number of reported cases shows that the shepherds' diagnoses are reasonably accurate where superficial lesions occur but that they probably miss some cases in which there are internal abscesses only.

An estimate of the percentage of lambs which developed tick pyaemia in the two districts is given in the following table:-

TABLE I. /

Year /

TABLE I.

	Approximate lamb crop.	Percentage of tick pyaemia cases.		
Year		1944	1945	1946
Kirkconnel	550	2.6%	7.2%	2.7%
Loch Lomond	2,500	-	3.7%	1.3%

Estimates of the ages, when first reported, of 54 cases of tick pyaemia were made. The results are given in the following table:-

TABLE II.

Age in weeks.	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to7
Number of cases.	1	11	16	16	9	1

Mean = 3.8 weeks. Standard deviation = $\pm$ 1 week.

Of 47 cases on Loch Lomondside in which the sex was noted during 1945 and 1946, 30 were males and 17 were females. The following table gives these figures compared with the total numbers of male and female lambs on the same farms, counted at ear-marking time, in these two seasons:-

TABLE III.

	Males	Females	Totals.
Pyaemia cases	30	17	47
Normal lambs	2,104	2,184	4,288
Totals	2,134	2,201	4,335

$\chi^2 = 4.054$ P = 0.05 to 0.02.

Thus in this district there appears to be a significant difference in sex susceptibility to tick pyaemia, ram lambs being more liable to be affected than /

than ewe lambs.

According to the shepherds, cases of tick pyaemia, in both districts, are mainly encountered in the period between the end of lambing (about the 14th of May) and ear-marking (about the 14th of June). Personal experience of cases over two seasons confirmed this observation. The earliest case seen was at Kirkconnel on the 7th of May. The majority of cases in both districts occur during the last week in May and the first few days in June. After ear-marking the shepherding is much less intensive and cases may occur which are not noticed. Such cases are few in number.

(b) The degree of tick infestation.

A series of counts of adult female ticks attached to ewes during the tick season was made in each district. Random samples of about 20 ewes were drawn from the flock on each occasion, and the ticks attached to the thighs, axillae and head were counted.

Similar counts of the ticks on lambs at the height of the pyaemia season were also made.

The following tables give the results of these examinations:-

TABLE IV. /

TABLE IV.

Tick counts on ewes at Kirkconnel.

Date.	Number of ewes in sample.	Mean number of ticks on each ewe.	Standard deviation of mean.
27/3/44	23	23.0	±12.13
12/4/44	23	44.3	±13.90
24/4/44	19	18.6	± 4.42
8/5/44	23	8.6	± 5.11
24/5/44	23	7.1	± 5.08
5/6/44	23	1.8	± 1.71
19/6/44	9	0.2	± 0.44
22/9/44	15	0.4	± 0.85

TABLE V.

Tick counts on lambs at Kirkconnel.

Date.	Number of lambs in sample.	Mean number of ticks on each lamb.	Standard deviation of mean.
8/5/44	16	3.3	± 2.27
24/5/44	21	3.0	± 2.14
5/6/44	20	2.5	± 2.14
19/6/44	22	2.1	± 1.53

TABLE VI.

Tick counts on ewes at Sallochy.

Date	Number of ewes in sample.	Mean number of ticks on each ewe.	Standard deviation of mean.
12/10/45	20	20.9	± 8.68
14/3/46	20	3.7	± 4.04
3/4/46	20	93.8	±71.68
24/4/46	20	77.0	±37.00
22/5/46	20	31.0	±25.00
14/6/46	20	3.5	± 2.11

TABLE VII.

Tick count on lambs at Sallochy.

Date.	Number of lambs in sample.	Mean number of ticks on each lamb.	Standard deviation of mean.
29/5/46	12	11.2	± 7.3

Although these series of tick counts were made in different seasons in the two districts, observations made while handling the sheep confirm the impression that tick infestation is higher on Loch Lomondside than at Kirkconnel, and further that there is an autumn increase of tick activity on Loch Lomondside which is absent at Kirkconnel.

(c) The seasonal variation of staphylococcal antibodies in the sera of ewes.

Blood samples were taken at intervals throughout a year, from random samples of ewes at Kirkconnel and Sallochy, and from a flock of ewes maintained at Moredun Institute, which is tick-free. The sera from these bloods were tested for α and β staphylococcal anti-haemolysins. The means of the titres at each bleeding are given in the following tables : -

TABLE VIII. /

TABLE VIII.

Seasonal variations of α anti-haemolysins in International Standard units per c.c. of serum.

Season		Moredun	Kirkconnel	Sallochy
Winter 1945.	Mean S.D. N.	2.7 ± 3.84 60		
Late Spring 1945	Mean S.D. N.	2.1 ± 2.13 38	4.5 ± 5.9 15	5.3 ± 5.9 16
Autumn 1945	Mean S.D. N.	3.8 ± 4.36 42	2.2 ± 1.67 20	5.2 ± 3.86 20
Winter 1946	Mean S.D. N.	2.4 ± 3.82 43	1.3 ± 1.56 20	1.0 ± 1.17 20
Late Spring 1946	Mean S.D. N.	2.5 ± 2.32 26	2.3 ± 2.44 15	3.4 ± 3.82 20

TABLE IX.

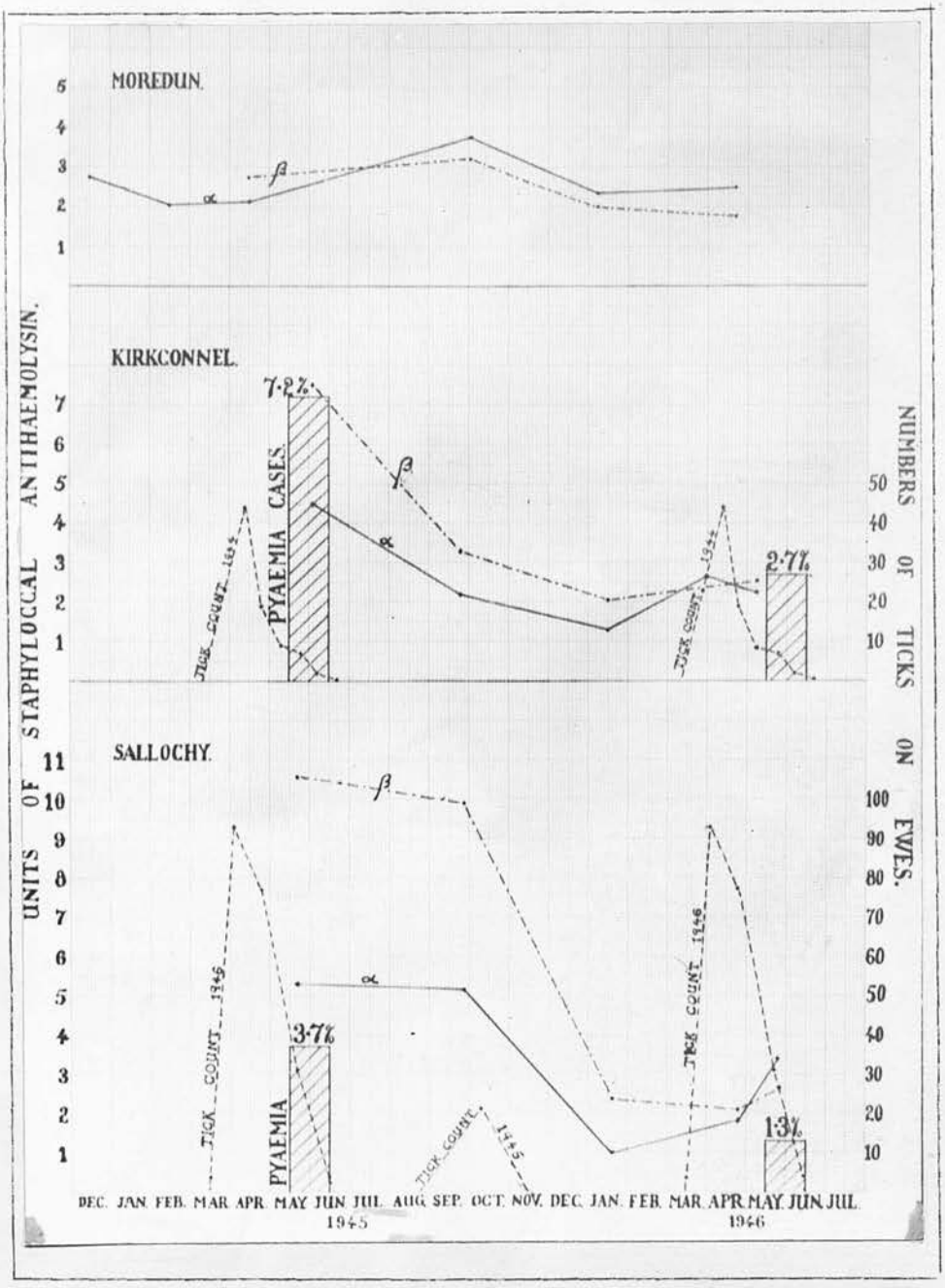
Seasonal variations of β anti-haemolysins in International Standard units per c.c. of serum.

Season		Moredun	Kirkconnel	Sallochy
Late Spring 1945	Mean S.D. N.	2.8 ± 3.52 38	7.5 ± 4.73 15	10.6 ± 9.11 16
Autumn 1945	Mean S.D. N.	3.2 ± 2.87 45	3.5 ± 2.65 20	9.5 ± 8.89 19
Winter 1946	Mean S.D. N.	2.0 ± 2.29 46	2.1 ± 1.82 20	2.5 ± 2.33 20
Late Spring 1946	Mean S.D. N.	1.8 ± 1.73 26	2.5 ± 2.61 15	2.7 ± 5.21 20

These tables show that, apart from a slight rise in autumn, the titres of the ewes at Moredun remained constant throughout the period of observation.

The/

Figure 1.



The seasonal variations of the mean of the staphylococcal anti-haemolysin titres of ewes, the incidence of tick pyaemia in lambs and the tick count in ewes.

The titres of the Kirkconnel ewes were raised in late spring, declined through autumn and winter and tended to rise again the following spring. The titres of the Sallochy ewes were raised in late spring, remained elevated during autumn, dropped in winter and showed the same tendency to rise again the following spring.

Figure 1. is a graphic representation of the following data :-

1. The seasonal variations of the means of the titres of staphylococcal anti-haemolysins in the sera of ewes at Moredun and at the two tick-infested farms.
2. The incidence of tick pyaemia in the two tick-infested districts.
3. The tick count on ewes. (Although only one curve representing the tick count is available for each farm, this has been repeated on the graph to show the approximate season and degree of tick infestation).

Discussion.

These results show that, while this form of pyaemia is a disease of lambs from 1 to 6 weeks old with a maximum incidence in lambs of 3 to 5 weeks old, there is also a well defined period from the middle of May to the middle of June in which cases occur.

The lambing period in both districts studied, extends from the middle of April to the middle of May.

A study of the curve of the tick counts on ewes given in Figure 1. shows that the tick infestation is heaviest/

heaviest before lambing starts and diminishes steadily throughout the lambing period. Observations made on flocks in Northern Ireland, where lambing was carried out on tick-free pastures, the lambs being subsequently moved to tick-infested pasture, showed that cases of tick pyaemia may occur in about 10 days after the start of tick infestation. It is therefore surprising that a greater number of the early lambs which are exposed from birth to the heaviest tick infestation do not develope pyaemia at the end of April and the begining of May.

A possible explanation of this apparent delay in the season of maximum incidence of the disease is the fact that, although the tick infestation is dropping throughout the lambing period, as lambing progresses the number of lambs exposed to infection rises to reach a maximum when all the ewes have lambed, i.e. about the 14th of May.

Figure 1. also shows that while the degree of tick infestation on Loch Lomondside is considerably greater than at Kirkconnel the incidence of pyaemia is smaller. The validity of this comparison is considerably reduced by the fact that different breeds of sheep are kept in the two districts with possible differences in breed susceptibility.

Further evidence that the tick is responsible for the infection of lambs with the causal staphylococcus in this pyaemia may be obtained from study of the anti-haemolytic titres of ewes. As is shown in Figure 1./

Figure 1., the mean α and β titres of ewes at Sallochy were raised following the spring tick season, they remained elevated during the autumn tick season and fell in winter. The titres of ewes at Kirkconnel were raised after the spring tick season and declined through autumn and winter. There is no autumn tick season at Kirkconnel. The titres of ewes at Moredun which is tick-free show no such variations. Thus, during seasons of tick activity, the ewes on tick-infested pastures receive staphylococcal antigen. It appears likely that this antigenic factor is a staphylococcal infection inoculated by tick-bite.

There is also some correlation between the height of the titres in ewes and the number of tick pyaemia cases. More cases of pyaemia occurred in both districts in 1945 than in 1946, and in both districts the rise in titre of the ewes' sera was greater in 1945 than it was in 1946.

Much further work is required on factors influencing the seasonal incidence of tick pyaemia. It might be shown that the rate of decline of the spring tick infestation, which is influenced by climatic factors, had a bearing on the number of tick pyaemia cases occurring.

PART II.

ON THE SOURCE AND MANNER OF INFECTION IN TICK PYAEMIA.

Although the occurrence of tick pyaemia is invariably associated with a tick infestation of the lambs, the rôle played by the tick is obscure and the following possibilities have to be considered : -

(1) The tick may act as a true vector of the disease. If this be the case, a staphylococcal infection acquired by the tick while feeding on a lamb suffering from pyaemia, is retained in the tick's body through the moulting period to the next instar. This internal infection is inoculated into a lamb in the following season when the tick engorges.

(2) The cuticle of the tick may carry the infection or the skin of the lamb may be infected; in either case the infection gains entrance to the tissues of the lamb by means of the wound caused by the tick while feeding.

(1) The Tick as a Vector of Tick Pyaemia.

In order to test the first hypothesis it was necessary :

(a) To find a means of sterilising the cuticle without causing injury to the tick;

(b) To produce an internal infection of the tick by feeding it on a host suffering from a staphylo-

coccal/

coccal septicaemia;

(c) To demonstrate that internal infection of the tick had occurred;

(d) To maintain suitably infected live ticks and examine them at intervals to determine whether the staphylococci within the tick remained viable until the tick had reached the next instar and was ready to attach and feed on a fresh host. If this were demonstrated and if the disease could be reproduced by feeding such experimentally infected ticks on lambs, the rôle of the tick as a true vector would be established.

Engorged females of Ixodes ricinus were collected from sheep and maintained under suitable conditions in the laboratory. The progeny of these ticks were used in the following experiments. (The stock was maintained by allowing the instars, which were not required for experiment, to engorge on hedgehogs.).

(a) The sterilisation of the cuticle of the tick.

Cetavlon has been shown to be an effective agent for the sterilisation of human skin (Williams et al, 1943 & 1944.). These workers found that Cetavlon in a dilution of 1/218,700 inhibits the growth of Staphylococcus aureus after incubation for 18 hours; and that in a culture which originally gave a colony count of 130,000, eight minutes exposure to a 1/10,000 concentration of Cetavlon reduced the count to 12.

Staphylococci isolated from a case of tick pyaemia, when/

when suspended in a 1/256,000 solution of Cetavlon for 30 minutes followed by three washings in sterile saline solution, failed to grow on inoculation into nutrient broth.

Engorged ticks of all three instars, washed in a 1 per cent solution of Cetavlon for 30 minutes followed by three rinsings in sterile water, have been observed to complete the next stage of their life-cycle. The available data suggests that the mortality in ticks so treated is no greater than in untreated ticks.

An engorged larval tick maintained in a 1 per cent solution of Cetavlon remained alive for six weeks. A 1 per cent solution of Cetavlon, therefore, is non-toxic or of low toxicity for ticks.

Thirty engorged larvae were washed in water; ten of them were ground up in a mortar with 5 c.c. of saline, 0.2 c.c. of the resulting emulsion was plated on sheep blood agar. No growth of staphylococci was observed. The remaining twenty ticks were transferred for 30 minutes to a No. 8 Brown's scale suspension of Staphylococcus aureus. They were then washed in 25 c.c. of distilled water. Ten of the larvae were ground and plated as above. 0.2 c.c. of the tick emulsion gave a growth of 150 colonies of Staphylococcus aureus. The remaining ten larvae were transferred to a 1 per cent solution of Cetavlon for 30 minutes, washed three times in distilled water, ground up and plated. No growth of staphylococci was observed.

This test shows that the cuticle of ticks

contaminated by a culture of staphylococci may be effectively sterilised by washing for 30 minutes in a 1 per cent solution of Cetavlon.

(b) The production of an internal staphylococcal infection in experimentally fed ticks.

The method of obtaining batches of infected ticks was as follows :- A batch of unengorged larvae or nymphs was liberated in a cloth bag into which a guinea-pig was placed. The guinea-pig was left in the bag overnight to allow time for the ticks to attach. The following morning the guinea-pig was transferred to a cage with open wire flooring standing over a tray containing water. As the ticks engorged and dropped off the guinea-pig, they were collected from the water. On the 2nd or 3 rd day after the ticks had been applied to the guinea-pig, the latter was given an intracardial injection of a suspension of Staphylococcus aureus sufficient to produce a septicaemia lasting from 3 to 6 days (McDiarmid 1946 c.). A septicaemia of this nature was obtained by using an inoculum of 0.05 c.c. of a No. 8 Brown's scale suspension of either strain 6591 or strain 1468. (Strain 6591 was isolated from the mouth of a ewe. Strain 1468 is the same strain after passage through a guinea-pig. These two strains appear to be identical in virulence and other characteristics). The inoculum was made up to a volume of 0.5 c.c.. The mean period of survival of 21 guinea-pigs, after inoculation with this dose, was 4.1 days $\pm$1.83 days. As larval and nymphal ticks take from 3 to 7 days to engorge/

it was thus possible to ensure that the majority of the ticks completed their engorgement after the guinea-pig had been infected. The engorged ticks were collected daily and a sample of each collection was examined for the presence of staphylococcal infection.

In early experiments groups of ten ticks, washed in Cetavlon and then in sterile water, as described above, were ground up in 5 c.c. of sterile saline and 0.2 c.c. of the tick suspension was plated on blood agar. The plates were incubated over-night and a count was made of the colonies of staphylococci. As the numbers of staphylococci in different samples from the same collection of larvae were found to vary greatly, in later experiments individual ticks instead of composite samples were examined bacteriologically. The engorged ticks were treated as before but each tick was ground up with sterile precautions on a glass plate. The resulting smear on the glass plate was emulsified in a loopful of sterile saline and stroked on to a sheep blood agar plate which, after over-night incubation, was examined for staphylococci.

(c) The demonstration of internal infections in experimentally fed ticks.

Single ticks from eight batches of engorged larvae that had been fed on infected guinea-pigs were examined at the time of their collection with the following results :-

TABLE X. /

TABLE X.

The numbers of larvae showing internal staphylococcal infections after feeding on infected guinea-pigs.

Total number of larvae recovered from each guinea-pig.	Number of larvae examined after infection of guinea-pig.	Number of larvae infected.	Percentage of larvae infected.
865	47	5	11
217	103	56	54
448	72	40	57
105	25	3	12
243	19	9	47
42	20	3	15
467	60	32	53
566	53	28	53

The mean of the percentages of infected larvae from these eight experiments is 38% with a standard deviation of ±20%.

(d) The survival of staphylococcal infection in experimentally fed ticks.

Other ticks from the same batches of larvae were allowed to develop and were examined from one to eight months after engorgement, but none showed the presence of staphylococci.

The following table gives the results of the examination of a group of larvae obtained from one of the infected guinea-pigs mentioned above :-

TABLE XI. /

TABLE XI.

Date.	Time.	Number of larvae collected.	Number of larvae examined.	Number of larvae infected.
2/10/44	3 p.m.	Larvae applied to guinea-pig.		
5/10/44	10 a.m.	3	3	0
	4 p.m.	109	24	2*
6/10/44	10 a.m.	32	10	0
	12 noon.	Guinea-pig infected.		
	4 p.m.	114	24	12
7/10/44	10 a.m.	25	12	10
8/10/44	12 noon.	112	24	14
9/10/44	10 a.m.	27	12	4
	2 p.m.	Guinea-pig killed in extremis.		
Totals after infection		278	72	40

* The staphylococcal infection in these larvae is an example of a naturally occurring skin infection being picked up by the larvae during engorgement as is described later.

Besides the ticks examined bacteriologically 116 nymphs were obtained from this batch of larvae. In June 1945, seven months after collection, these 116 nymphs were washed in Cetavlon and in water and examined for the presence of staphylococci but none was found to be infected.

The ticks in these experiments had been maintained at a temperature of 24°C. in order to hasten the moulting process. It appeared possible that this temperature, which is abnormally high compared with field conditions, was responsible for the failure of

the/

the staphylococci to survive the moulting period. A batch of larvae after engorging on an infected guinea-pig was retained in the cold-store at a temperature of 4°C.. Samples of these larvae were examined periodically for staphylococcal infection. The results of this experiment were as follows :-

TABLE XII.

Date.	Number of larvae collected.	Number of larvae examined.	Number of larvae infected.
20/8/45	Larvae applied to guinea-pig.		
22/8/45	252	20	0
	Guinea-pig infected.		
23/8/45	61	10	6
24/8/45	238	39	18
25/8/45	15	4	4
	Guinea-pig dead.		

Examination of samples of larvae taken at intervals from the batch of larvae collected on the 24th of August gave the following results :-

TABLE XIII.

Date.	Number of larvae examined.	Number of larvae infected.
24/8/45	39	18
22/9/45	20	3
1/11/45	20	1
30/11/45	21	0
11/1/46	20	0

It thus appears that a staphylococcal infection persists/

persists for a relatively short time, less than three months, in the body of the engorged larval tick even when the larvae are kept at low temperatures; and it is unlikely that, under field conditions, larval ticks which become infected by feeding on lambs suffering from a staphylococcal septicaemia will retain the infection into the next instar. According to Campbell (unpublished) ticks under natural conditions usually take a year to complete each instar.

A much smaller proportion of the number of nymphs applied to guinea-pigs became attached and engorged, and smaller numbers of engorged nymphs were available for experiment. The results of bacteriological examination of nymphs fed on seven infected guinea-pigs and examined at the time of collection were as follows :-

TABLE XIV.

Total number of nymphs from each guinea-pig.	Number of nymphs examined after infection of guinea-pig.	Number of nymphs infected.	Percentage of nymphs infected.
18	7	2	29
17	5	1	20
40	12	4	33
8	1	1	100
16	2	2	100
43	10	1	10
11	4	0	0
153	41	11	27

Fifty-three of the remaining nymphs from these batches/

batches were examined at intervals of from one to five months after engorgement, but none of these was infected.

It would thus appear that a varying percentage of nymphs, fed on guinea-pigs suffering from a staphylococcal septicaemia, become infected; but, as in the case of larvae, this infection is not retained through the moult to the following instar.

The examination of ticks from pyaemia cases.

Engorged ticks collected from cases of tick pyaemia are occasionally found to be infected with staphylococci, but the incidence of such infections is not high. Of a series of 19 engorged females and 5 males collected from cases only one was found to be infected.

The examination of ticks from pasture.

Un-engorged ticks were collected by dragging a blanket over the pasture on farms where tick pyaemia is prevalent. These were examined for staphylococci by washing in Cetavlon and plating individually as described above. In all, 362 nymphs, 5 females and 5 males were examined from this source. None of them was found to be infected.

Conclusions.

The above experiments are strong evidence that the tick does not act as a true vector in tick pyaemia, and that if tick-bite is the mode of entry of the staphylococcus into the lamb's body the tick merely acts as an inoculator of an infection derived from some source outside its own body.

The Tick as an Inoculator of External Infection.

As the most probable source of the causal organism of tick pyaemia appeared to be a skin infection of the lamb, experiments were designed to determine whether skin infections of staphylococci occur in lambs and whether the organisms present in these infections are similar to those isolated from cases of tick pyaemia.

The determination of staphylococcal infection in ewes and lambs by the examination of swabs.

A study was made of infections of haemolytic staphylococci in the natural orifices and skins of ewes before lambing, and on the skin of their lambs after birth.

Animals.

A flock of 19 Blackface ewes was purchased from a tick-free hillfarm and kept at Moredun. Swabs were taken from these ewes on the following dates :- November 1943, 24/1/44, 21/2/44, 21/3/44, 4/4/44, 19/4/44, 2/5/44 and 15/5/44. Swabs were also taken from each ewe within 24 hours of lambing. Lambing started on the 19th of March and finished on the 31st of May. Skin swabs were taken from the lambs shortly after birth and at intervals of 14 days thereafter.

Twenty-four Cheviot ewes on The Knowe, Kirkconnel, Dumfriesshire, - a tick-infested farm where pyaemia occurs - were eartagged and swabs were taken on the following dates :- 2/2/44, 28/2/44, 27/3/44, 12/4/44, 24/4/44, 8/5/44 and 24/5/44. Lambing started about the 15th of April and finished about the 15th of May. Skin

swabs were taken from the lambs at intervals of 14 days from within 13 days of birth until the 5th of June.

Methods. The following method of taking swabs was carried out in all cases :-

Ewes: 1. Mouth - a sterile swab was passed into the mouth, between the molar teeth and the cheek, and withdrawn.

2. Nose - a sterile swab was passed up the left nostril and withdrawn.

3. Skin of udder - a sterile swab was dipped in sterile saline solution and rubbed over an area about 1" square on the surface of the udder. It was then passed over the point of one teat.

4. Skin of thigh - a sterile swab moistened with saline was rubbed on the inner surface of the thigh.

5. Vagina - the lips of the vulva were opened and a sterile swab was passed into the vagina and withdrawn.

Lambs: Four sterile swabs moistened with saline were rubbed on the hairless skin of the axillae and insides of the thighs.

Examination of swabs. The swab was transferred from the swab tube to a tube containing 5 c.c. of Hartley broth and allowed to soak at room temperature for approximately 2 hours. The swab was then thoroughly shaken in the broth and removed. A series of dilutions decreasing by 1/10 in saline was made from the broth. 0.2 c.c. of the broth

and/

TABLE XV.

EXAMINATION OF SWABS FROM EWES AND LAMBS. AT MORTEDUN.

No. of Ewe.	Nov. 1943	24/1/44	21/2/44	21/3/44	4/4/44	19/4/44	2/5/44	15/5/44	Date of lambing.	Ewe at lambing.	Lamb at birth.
649	N 11 88	N 8 UT	M 7 X2	-	-	-	-	-	3/5/44	-	-
650	N 7 X2	N 8 X2	N 1 UTB	M 1 (UTB X2	M.12 UTB	N 1 UTB	-	-	11/4/44	-	-
652	-	M 8 S7	M 54 S7	M 13 X2	-	-	M 16	-	11/4/44	-	-
653	-	M 5 X2	-	-	-	-	-	-	12/5/44	-	-
654	N 15 X2	-	M 6	M 6 UT	M 4	N 1 X2	-	-	19/3/44	M 6	-
655	-	M 1	-	-	-	-	-	θ	26/3/44	-	-
656	V 36000 X2	V 20 X2	M 27 X2	-	-	-	M 100 X2 U 3 X2	-	11/4/44	M 1	-
658	-	V 700 X2	V 900 X2	V 1 X2	-	-	-	-	31/5/44	-	-
659	M 13 X2 N 50	M 15 X2	-	V 1 X2	M 2 X2	M 1 X2 T 1 X2	M 2 X2	M 31	10/4/44	T 1	-
660	-	-	-	-	-	M 1 UT	M 49 UT	M 12	13/5/44	M 12	LT 1UT
661	N 1500 X2 V 100000 X2	M 1 88	N 1 X2	M 6 X2	-	-	U 80 X2	-	10/4/44	-	-
662	M 40 X2	M 1 X2	-	-	-	-	M 1 X2	M 1	31/3/44	-	-
664	-	-	-	-	-	-	-	-	13/5/44	-	-
665	N 50000 X2 U 1300 X2	N 70 X2 M 5 X2	N 380 X2 M 2 X2	-	-	-	-	-	16/4/44	-	-
666	-	-	-	-	M 1 UT	-	-	-	16/4/44	-	-
668	-	N 1 X2	-	-	-	-	-	-	3/5/44	-	-
670	-	-	M 5 88	-	-	-	M 10 X2	M 24	Not in lamb.		
671	M 100 S7	-	M 3 X2	M 50	-	M 3 X2	M 100	M 11	15/4/44	M 3	-
672	-	M 60 X2 N 4000 X2	M 13 X2 N 8 X2	-	M 100 X2 N 2 X2	M 60 X2 N 15 X2	M 100 X2	M 5	Not in lamb.		

No. of Lamb.	4/4/44	19/4/44	2/5/44	15/5/44
756	θ	θ	θ	-
died	twinned from ewe 659 743	-	-	-
747	θ	LT 1 X2	Expt.	-
765	θ	θ	θ	-
739	-	-	-	-
740	-	-	-	-
741	-	LA 7 X2	-	-
746	-	LT 7 X2	Expt.	-
768	θ	θ	θ	θ
743	To ewe No. 650.			
744		-	Expt.	Expt.
766	θ	θ	θ	LT 1 UT
745	θ	RT 1 X2	-	Expt.
742	-	LT 1 X2	-	-
767	θ	θ	θ	-
750	θ	-	RT 2	-
751	θ	-	-	-
758	θ	θ	-	-
748	θ	-	-	-
749	θ	-	-	-

Abbreviations:-

M = mouth
N = nose
U = skin of udder
T = " " thigh
V = vagina
Number = number of haemolytic staphylococci in 0.2 cc of broth.

RA = Right axilla
LA = Left axilla
RT = Right thigh
LT = Left thigh
Expt. = Experimental infection.
- = No staphylococci detected.
θ = Animal not examined.

X2 = Phage type.
88 = " "
S7 = " "
UTA = " "
UTB = " "
UT = Untypeable.

and 0.2 c.c. of each dilution in the series was sown on to a series of 5% sheep blood agar plates. The plates were incubated over-night, allowed to cool for 1 hour at room temperature and the colonies of haemolytic staphylococci were counted. Cooling after incubation facilitates the identification of the α/β haemolysis shown by the majority of the staphylococci encountered.

The weight of infection in positive swabs, as given in the tables, is an estimate of the number of colonies obtained from plating 0.2 c.c. of the Hartley broth on a blood agar plate. This estimate was arrived at by multiplying the number of staphylococcus colonies on each plate of a series by its respective dilution and taking the mean of the answers.

A subculture from most of the strains isolated was sent to Mr. Williams Smith, London School of Hygiene and Tropical Medecine, who examined them for their bacteriophage type.

Results.

The results are set out in tables XV and XVI.

Ewes: Study of these tables indicates that there was a tendency for more ewes on both farms to carry staphylococcal infections during the winter months than in spring. The number of heavy infections is also greater in winter than in spring.

At lambing five of the seventeen Moredun ewes, which lambed, were infected; and at the first swabbing after lambing thirteen of the twenty-two Kirkconnel ewes, which lambed, were infected. This difference is not significant ($\chi^2 = 3.399$, P = 0.1 to 0.05).

None/

TABLE XVI.

EXAMINATION OF SWABS FROM EWES AND LAMBS AT KIRKCONNEL.

No. of Ewe.	Ewes. 2/2/44	28/2/44	27/3/44	12/4/44	24/4/44	8/5/44	24/5/44	No. of Lamb.	Lambs. 24/4/44	8/5/44	24/5/44	5/6/44	19/6/44
832	-	-	-	-	M 1 X2	* -	θ	464	464 θ	-	RA 1 S7	-	θ
833	M 110 UTA N 3000 UTA	-	M 1 UTA	-	-	* -	θ	462	462 θ	-	-	-	θ
834	M 30 X2 N 3300 S7	M 50 S7 N 10 S7	Ewe died.										
835	-	-	-	-	-	* -	θ	461	461 θ	-	Escaped during swabbing.		A 20 S7
836	-	-	-	-	M 1 X2	* -	θ	465	465 θ	-	RT 3 X2	-	θ
837	M 18 X2	M 1 X2	-	M 15 X2	M 3 X2	* M 6	θ	526	526 -	-	RA 1 X2	-	θ
838	M 13 88 N 53 88	-	-	-	* M 7 88	-	θ	458	458 θ	-	-	-	θ
839	M 1 88 N 21 88	-	-	M 1 88	-	-	* -	894	894 θ	θ	-	θ	θ
840	M 8 X2 N 350 X2	-	M 2 X2	-	M 18 X2	* M 20 X2	θ	459	459 θ	-	-	-	θ
841	M 13 88 N 1 88	M 2 88	M 4 UTA	-	M 3 88	-	Not in lamb.						
842	M 12 X2	-	M 1 X2	-	* -	-	θ	437	437 RA 1 X2	-	-	RA 50 X2 LA 2 X2	θ
843	M 25 N 300 X2 U 1 UT	M 12 X2 N 80 X2	M 2 X2	-	M 3 X2	M 9	* M 7 X2	994	994 θ	θ	-	RA 10 X2 LA 1 X2 RT ½ LT 1 S7	θ
844	N 7 UTA	-	-	-	* M 2 UTA	M 6 X2	M 1	468	LT 2 UTA	LA 3	LA 1	A 1 X2	
845	M 45 X2 N 10000 V 2 X2	N 1 X2 V 163 X2	M 24 X2 V 900 X2	M 8 X2	-	M 21 X2 N 1	* N 1 X2 V 1 X2	77	468		RT 2 88 LT 78 S7	RA 1 X2 LT 1 X2	θ
846	-	-	-	-	-	* -	θ	532	77 θ	θ	-	-	θ
847	M 400 X2 N 11 X2	-	M 1 X2	M 14 X2	-	* M 5 X2	θ	463	532 θ	-	-	-	θ
848	M 10 UTA	M 2 UTA	M 5 UTA	-	N 1 UTA	* -	θ	466	463 θ	-	-	-	θ
850	-	-	-	-	-	-	* N 2	992	466 θ	-	-	-	θ
851	V 10 88	-	-	-	-	* A 300 88	θ	531	992 θ	-	-	-	θ
852	M 42 UTB N 2 UTB V 87 88	M 4 UTB	M 10	-	V 2 88	M 13 V 15	* U 7 X2 V 6 88	993	531 θ	θ	-	A 300 X2	-
853	M 280 88 N 53 88 V 2 UTA	V 6 UTA	-	-	M 3 88 V 1	* V 1 UTA		460	993 θ	θ	-	LA 1 X2	θ
854	-	-	-		* M 1	-	θ	438	460 θ	-	RA 1 LA 9 UTA	-	θ
855	M 2 V 41	-	-	M 1 X2	-	* M 6 X2	θ	467	438 LA 10	-	-	-	-
856	-	-	-	M 3 UTA	M 8	M 196 M	* M 26 UTA	991	467 θ	-	RA 3 UTA LA 15 88	LA 1 88	θ
									991 θ	θ	-	-	θ

Abbreviations - as in Table XV.

A = tick abscess.
* = First swabbing after lambing.

None of the infections at the first swabbing after lambing was heavy. The figure A 300 in ewe 851 is is the count of staphylococci from an abcess following tick-bite and is omitted from the above comparisons.

As is shown in the tables, when infections were detected in individual ewes on several different occasions, they were usually of the same bacteriophage type.

Lambs: Seven out of nineteen lambs born at Moredun and ten out of twenty-two lambs from eartagged ewes at Kirkconnel showed a naturally occurring skin infection. This difference in incidence is not significant ($\chi^2 = 0.311$, $P = 0.7$ to 0.5). The staphylococcal infections in tick abcesses (lambs Nos. 461 and 531) are again omitted from this comparison. Swabs were taken from several other tick abcesses but these failed to show staphylococcal infection and are not recorded.

The following table gives the distribution of infection in lambs in relation to infection in their dams at lambing :-

TABLE XVII.

Farm.	Ewe not infected.		Ewe infected.	
	Lamb not infected.	Lamb infected.	Lamb not infected.	Lamb infected.
Kirkconnel	6	4	6	6
Moredun	8	5	4	2
Totals	14	9	10	8

There is no correlation between the presence or absence of infection in the ewes and in their lambs ($\chi^2 = 0.118$, $P = 0.8$ to 0.7).

If/

If however the 'phage types isolated from lambs are compared with those isolated from their mothers it is found that on 18 occasions the type present on the lambs skin was the same as a type which had been isolated from the mother, while on seven occasions the types were different. Unfortunately type X2 is of such common occurrence that the exact source of an infection with this type cannot be definitely decided.

Incidence of tick pyaemia. None of the lambs in this experiment developed pyaemia.

The incidence of skin infections in tick pyaemia cases compared with that in normal lambs.

The incidence of staphylococcal infections in skin swabs from 19 cases of tick pyaemia was compared with the incidence in skin swabs from 87 normal lambs of susceptible age on tick infested farms with the following results :-

TABLE XVIII.

Lambs.	Skin infected.	Skin not infected.	Totals.
Pyaemia cases.	9	10	19
Normal lambs.	20	67	87
Totals.	29	77	106

$\chi^2 = 4.663$, P = 0.05 to 0.02.

Thus there appears to be a significantly greater number of skin infections in lambs affected with pyaemia than in lambs not so affected.

Conclusions.

These experiments show that infections of haem-

olytic/

haemolytic staphylococci occur in the natural orifices and on the skin of ewes and also on the skin of lambs.

The infections on lambs' skins may be derived from their mothers but infections from other sources may also occur.

There appears to be little difference in the incidence of the infections in tick-free and tick-infested farms.

Skin infections are more prevalent in lambs suffering from pyaemia than in normal lambs.

A comparison of haemolytic staphylococci from external infections in ewes and lambs and the haemolytic staphylococci from tick pyaemia cases.

A bacteriological comparison of 211 strains of haemolytic staphylococci obtained from ewes and lambs, as described in the previous section, and 56 strains of staphylococci isolated from cases of tick pyaemia in lambs was carried out.

Methods.

During 1944 the strains, after isolation were sown on to Hartley agar slopes in McCartney bottles, incubated over-night and retained at room temperature until examined. In 1945 Worth's medium was substituted for Hartley agar. The seeded cultures were not incubated but were stored at room temperature. Before examination a sub-culture was made on to Hartley agar and incubated for 18 hours. The growth was washed off into

2 or 3 c.c. of sterile saline and used for the following tests :-

Pigment production. - Loopfuls of culture suspension were stroked on to plates of a medium containing 33% sterile milk added to Hartley agar. The plates were incubated at 37°C for 48 hours and stored at room temperature for 5 days. The pigments were then described.

Three classes of pigment were observed :-

(1) Golden - a deep orange-golden colour.

(2) Fawn - this class, which contains most of the strains recovered from sheep, ranges from a brownish-golden through fawns of diminishing intensity to just off-white colours. Considerable difficulty was experienced in differentiating between golden and the brownish-golden at one end of the range, and between white and off-white at the other.

(3) White - china-white.

Haemolysis. - Haemolysin production was examined by the method of Bryce and Rowntree (1936.) using a loopful of the culture suspension sown as a single drop on a 5% sheep blood agar plate. The plates were incubated for 18 hours and allowed to cool at room temperature for 2 hours. An attempt was made to gauge the degree of haemolysin production by measuring the widths of the clear (α) zone and the partial (β) zone of haemolysis, but these measurements were found to vary greatly with the same strain from plate to plate and also on different parts of the same plate.

Coagulase/

TABLE XIX.

The bacteriological examination of staphylococci from normal ewes and lambs and from tick pyaemia cases.

Source of strains.	Number of strains examined.	Coagulase.	Haemolysis.	Pathogenicity.	Pigment.	Bacteriophage type.
Swabs from ewes & lambs	211	+ 211	$\alpha\beta$ 209	+ 203	Fawn 178	X 2 100 88 23 UTA 13 UTB 4 S7 4 UT 5 M 29
					Golden 24	X2 4 UTA 5 UTB 3 S7 1 M 11
					White 1	S7 1
				- 6	Fawn 5	X2 1 88 1 S7 3
					White 1	X2 1
			α 1	- 1	Fawn 1	M 1
			β 1	+ 1	Fawn 1	X2 1
Tick pyaemia cases.	56	+ 56	$\alpha\beta$ 53	+ 53	Fawn 43	X2 17 88 12 UTB 1 S7 1 UT 4 M 8
					Golden 10	88 1 S7 3 420 1 M 5
			α 2	+ 2	Fawn 2	X2 1 88 1
			β 1	+ 1	Fawn 1	M 1

Abbreviations:- UT = untypeable. M = not submitted to phage typing.

Coagulase production. - Citrated rabbit plasma was diluted 1/5 with normal saline. 0.5 c.c. of the dilution was pipetted into test-tubes of 8 m.m. diameter. Each tube was inoculated with a loopful of the different culture suspensions under examination. On each occasion on which the test was set up control tubes containing diluted rabbit plasma only were included. The tubes were incubated for three hours and examined for coagulation. The tubes were allowed to stand on the bench over-night and were again examined. Positive coagulation was recorded if, at either of the readings, the tube could be inverted without loss of fluid.

Pathogenicity for mice. - A suspension equal in opacity to No. 8 of the Brown's scale was made from each culture to be tested. This suspension was then diluted 1/3 and 0.3 c.c. was injected intravenously into three mice. Deaths were recorded for 8 days after inoculation. When one or more mice survived the inoculation, the test on that strain was repeated. A positive result was recorded when more than half of the mice inoculated with the strain under test died within 8 days.

Bacteriophage type. - The bacteriophage type of the majority of the strains from both sources was determined by Mr. Williams Smith.

Results.

The results of these tests are set out in table XIX.

This shows that all the strains of haemolytic staphylococci from external infections in ewes and lambs, and from pyaemia cases are coagulase positive; the big majority/

majority produce $\alpha\beta$ haemolysins, are pathogenic to mice and produce fawn or golden pigment.

Of the bacteriophage types, X2, 88, S7 and UTB are common to both groups. These four types include 86% of the strains isolated from ewes and lambs, and 88% of the strains from cases of tick pyaemia. There is a smaller percentage of X2s and a greater percentage of 88s in the strains from tick pyaemia cases than in the strains from ewes and lambs.

Conclusions.

The close correlation between the bacteriological properties of the haemolytic staphylococci from external infections in ewes and lambs and those from cases of tick pyaemia is strong evidence that they are the same species.

This observation is confirmed by the fact that in 88% of the strains from pyaemia cases the bacteriophage types are the same as those in strains from ewes and lambs.

Attempts to produce pyaemia by feeding ticks on skin contaminated with staphylococci.

The experiments already described show that infections by staphylococci similar to those found in cases of tick pyaemia occur on the skins of lambs. The following experiments were carried out to determine whether pyaemia could be set up by feeding ticks on skin contaminated with staphylococcal culture.

Although naturally occurring tick pyaemia is confined to young lambs an attempt was made to produce the disease in guinea-pigs using a larval tick infestation.

Three groups of two guinea-pigs were treated as follows:-

Group 1. A cluster of larvae was applied to each pig by the method already described.

Group 2. The faces and ears of the pigs were rubbed with a swab soaked in a No. 8 Brown's scale suspension of strain 6591 and a cluster of larvae was applied to each.

Group 3. The face and ears of each pig were contaminated with staphylococci as above.

Samples of the engorged larvae, as they dropped off the guinea-pigs in groups 1 and 2, were examined individually, by washing in Cetavlon and plating on sheep blood agar, for internal infections of staphylococci.

On the 10th day after the start of the experiment/

experiment (i.e. after all attached larvae had dropped off) a swab was taken from the face and ears of each guinea-pig and examined for staphylococci.

The guinea-pigs were kept under observation for three weeks but none of them developed pyaemia.

The results of the bacteriological examination of skin swabs and engorged larvae are given in the following table:-

TABLE XX

Identification of guinea-pig	Group 1.		Group 2.		Group 3.	
	a.	b.	c.	d.	e.	f.
Number of larvae collected.	329	146	126	210	-	-
Number of larvae examined.	140	90	69	140	-	-
Number of larvae infected.	0	3	9	14	-	-
Percentage of larvae infected.	0%	3%	13%	14%	-	-
Number of staph. colonies from face swab.	0	2	63	N	N	N

N= numerous.

The strains of staphylococci isolated from the larvae off guinea-pig b. and from its face swab appeared to be identical. They both produced golden pigment and a small amount of α haemolysin. Both were coagulase + and both were pathogenic to mice. This strain was presumably a naturally occurring skin infection./

infection. Several such strains were encountered during the experiments on the artificial infection of ticks already described.

The strains isolated from the larvae and the face swabs from guinea-pigs c.d.e. and f. all gave the same reactions as the original contaminating strain (6591) i.e. they produced fawn pigment and α/β haemolysins, they were coagulase + and pathogenic to mice.

Experiments with lambs. As the above experiment failed to produce pyaemia in guinea-pigs, experiments were carried out on lambs of susceptible ages.

Considerable difficulty was experienced in getting ticks to attach to the area of contaminated skin. The following methods were tried:-

1. The ticks were placed in a cloth bag which was tied over the lamb's head for 12 hours. Very few ticks applied by this method attached, a possible explanation being that the raised temperature and increased moisture content of the atmosphere inside the bag, due to the lamb's respiration, inhibited the attaching process.

2. A flanged ring of tin, fitted with a wire-gauze lid, was attached to a shaved area on the lamb's back with resin and bees-wax. Owing to the curving of the back and to muscular movement few of the boxes, applied in this manner, remained on long enough/

enough for the ticks to attach.

3. Nymphs, contaminated by being kept in an agar slope culture of the staphylococcus at room temperature for 3 days, were dropped on to the thighs and groin of a lamb (745).

4. Ticks were applied to the scrotums of ram lambs in muslin bags retained in position by an elastic band. These bags usually stayed on long enough to allow the ticks to attach but did not remain on for the whole engorgement period.

5. The most satisfactory results were obtained by using a paste of gelatine, zinc oxide, glycerine and water (Neitz et al 1941). The wool was clipped from an area on the flank and, after the skin had been contaminated, a piece of butter muslin was fixed over the area by painting round the edges with the paste. Before the upper edge of the muslin was stuck on, the ticks were dropped into the space between the skin and the muslin. The paste was also used to fix bags, containing ticks, on the tail, scrotum and ears, paste being applied round the neck of the bag after this was in position. A successful attachment of ticks was obtained, in a ewe lamb by covering the insides of the thighs and the posterior part of the abdomen with a strip of muslin stuck round the edges with paste./

TABLE XXI.

The effect of feeding ticks on lambs' skin contaminated with staphylococci.

No. of lamb.	Age in days.	Sex.	Infecting strain.	Other diseases.	Ticks used.	Number of ticks seen attached.	Symptoms.	Local lesions.	Infection of local lymph-glands.	Generalised staphylococcal infection.
746	8	Male	6591	-	-	-	-	+	?	-
745	22	Male	6591	-	L nymphs	6 n	-	+	?	-
747	17	Male	6591	-	L nymphs	9 n	-	+	?	-
748	10	Male	6591	-	L adults	16 ♀	-	+	?	-
749	16	Male	6591	-	L nymphs	25 n	-	+	?	-
200	20	Male	1468	-	L adults	16 ♀	Unthrifty	+	+	Sterile abscess in liver.
201	21	Male	1468	T.B.F. *	F adults	18 ♀	Died on 16th day.	+	+	-
204	20	Male	1468	S.C.	L nymphs	77 n	Died on 10th day.	+	+	-
206	20	Male	1468	-	L larvae	100 l	-	+	?	-
209	27	Male	1468	P.C.	F adults F nymphs	6 ♀ 18 n	Died on 3rd day.	+	+	-
225	27	Female	S.4 S.11 C.10 OM 6. OM.8.	T.B.F.	L adults	10 ♀	-	+	-	Hepatic lymph-gland.
226	26	Female	S.4.S.11 C.10 OM 6 OM 8	T.B.F.	L nymphs F nymphs	15 n	-	+	+	Spleen liver lungs
227	42	Female	1468	T.B.F. *	F adults L nymphs	9 ♀ 0 n	-	+	-	-

Abbreviations:-

F = Field ticks.
L = Laboratory reared ticks.
♀ = Females.
n = Nymphs.
l = Larvae.

T.B.F. * = Tick-borne fever from ticks applied.
T.B.F. = Tick-borne fever from injection of infected blood.
P.C. = Lamb in poor condition at start of experiment.
S.C. = Generalised mixed infection of a streptococcus and a coliform organism.
? = Lymph-glands not examined.

paste.

Except in the case of lamb 745, when contaminated ticks were used, contamination of the skin was carried out by rubbing the area with a swab soaked in a suspension of an 18 hour culture of staphylococci. Strains 6591 or 1468 were used for infection on all the lambs except Nos. 225 and 226 when a mixture of strains from tick pyaemia cases was used.

Lamb 746 was the subject of a preliminary experiment on the contamination of skin without the application of ticks.

As tick-borne fever may be a factor which reduces the lamb's resistance to staphylococcal infection, lambs 225 and 226 were given an artificial infection of this disease. Lambs 201 and 227 developed an attack of tick-borne fever presumably acquired from the ticks collected in the field which were used on these lambs. In most of the other experiments the ticks used had been reared in the laboratory from the egg, the stages being fed on hedgehogs.

The results of these experiments are summarised in table XXI.

Lamb 746.

The groin and inner sides of both thighs were contaminated with strain 6591.

Small superficial abcesses developed on both thighs within 7 days of contamination. These abcesses resolved in 17 days. No other symptoms developed. Swabs taken on the 2nd, 7th and 13th day after contamination/

contamination showed heavy infections of α/β haemolytic staphylococci. Swabs taken on the 26th and 36th day after infection were negative.

Similar local lesions were seen in all the lambs in the skin contamination experiments. The intensity of the local reaction varied from a slight exematous exudate, which formed dry scabs on the wool, to cutaneous abcess formation.

Lamb 745.

Nymphs contaminated by keeping on a staphylococcal culture were dropped on to the groin region. Only six of these nymphs were seen to have attached. A blood agar plate sown from one of the unattached nymphs gave a heavy growth of staphylococci. On the 13th day after the application of the nymphs a swab from the left thigh yielded 5 colonies of a haemolytic staphylococcus similar to the contaminating strain. No symptoms of pyaemia developed and the lamb was not destroyed.

Lambs 747, 748, 749 and 206.

In these lambs ticks, as detailed in the table, were applied to contaminated skin. Four to six days after contamination swabs were taken from the contaminated skin areas and examined for haemolytic staphylococci. Heavy infections were found in all cases. None of these lambs developed symptoms of pyaemia and none were destroyed.

Lamb 200.

Laboratory reared adult female ticks were applied to the contaminated skin of the flank, tail and scrotum. Sixteen of these ticks attached. Five days after contamination heavy growths of staphylococci were obtained on swabs from the skin areas. This lamb became unthrifty and on the 14th day the temperature rose to 105°F and remained about that level until the lamb was killed. No symptoms of lameness developed and the lamb was destroyed on the 31st day after infection.

At post-mortem examination an old abcess, about ½" in diameter was found in the liver. The walls of the abcess were thickened and the cavity contained a thin yellow fluid. The abcess content and portions of the wall were sterile on cultivation. No staphylococci were detected on examination of sections. Four colonies of haemolytic staphylococci were isolated from the left precrural lymph-gland. Cultures from the viscera, other lymph-glands and from an old abcess in the skin of the tail were negative.

Lamb 201.

Field female ticks were applied to the same areas of skin as in lamb 200. The number of ticks attaching and the results of skin swabbing were similar.

On/

On the 6th, 7th and 8th day after application of the ticks, the lamb showed a temperature rise reaching 106.4°F.. Tick-borne fever was confirmed by finding inclusion bodies in the polymorphs in blood smears, and by passage of the disease by inoculation of blood into susceptible ewes. Progressive emaciation and transient stiffness were noted in this lamb. Death occurred on the 16th day after the start of the experiment.

On post-mortem examination the spleen was found to be enlarged and soft, the heart muscle was flabby and pale, and there was some excess of peritoneal fluid. These findings were taken to indicate heart failure which was probably due to the attack of tick-borne fever. Nine female ticks were attached to an area of about ½" in diameter on the ventral surface of the root of the tail. This lesion is discussed further in the section dealing with the pathology of tick-bite. Eight of these ticks were examined bacteriologically and all were found to be infected with staphylococci. Cultures from the lesion in the cutis were also positive. The staphylococcus was also isolated from the skin of the tail and scrotum, from an abcess in the tail distal to the site of tick attachment and from the internal illiac lymph-glands. Cultures from the viscera and blood/

blood were negative.

Lamb 204.

A good attachment of laboratory reared nymphs was obtained on the contaminated skin areas of this lamb. Abcesses developed on the tail and scrotum. There was a progressive loss of condition and symptoms of respiratory involvement developed. The lamb died on the 10th day after the start of the experiment.

On post-mortem examination the lungs were found to be congested. There was excess of peritoneal fluid and a slight flushing of the peritoneal membrane. Cultural examination of the viscera showed the presence of a mixed streptococcal and coliform infection in the liver, spleen, kidneys, lungs and internal illiac lymph-glands. Growths of haemolytic staphylococci were obtained from an abcess in the tail and from the right precrural lymph-gland. The cause of death was presumed to be the intercurrent infection from an unknown source.

Lamb 209.

This lamb was in somewhat poor condition at the start of the experiment. A mixture of field nymphs and field adults was applied to contaminated skin areas. The lamb died on the 3rd day of the experiment.

Post-mortem examination failed to show the cause of death. Cultures from the blood/

blood and viscera were negative. Numerous haemolytic staphylococci were obtained in culture from the local lesions on the tail, flank and scrotum and also from both precrural lymph-glands.

Lamb 225.

A subcutaneous injection of 5 c.c. of blood from a ewe, which had passed through an artificially produced attack of tick-borne fever, was given to this lamb. As a possible reason for the failure to transmit pyaemia to the earlier lambs in this series might be a low invasive power of strain 1468, lamb 225 was contaminated on the flank and tail with a mixture of 5 strains of staphylococci which had been isolated from pyaemia cases. Female ticks were applied. On the 4th to 8th days after the above operations a rise of temperature was recorded. Tick-borne fever was confirmed by the finding of inclusion bodies in blood smears. No other symptoms developed and the lamb was killed on the 20th day.

The only abnormality detected at post-mortem examination, apart from an abcess in the skin of the tail, was slight enlargement of the lymph-glands. A blood agar plate sown from the hepatic lymph-gland gave a growth of one colony of haemolytic staphylococci. Very numerous haemolytic staphylococci were isolated/

isolated from the tail abcess. Cultures from the viscera, blood and other lymph-glands were negative.

Lamb 226.

This lamb received an injection of infected blood from a tick-borne fever case as in lamb 225. Three days later the skin of the thighs and belly were contaminated with the same mixture of strains, and nymphal ticks were applied.

A temperature rise on the 3rd to the 7th days after the injection of blood showed that tick-borne fever had developed. This was confirmed by finding inclusion bodies in blood smears. No other symptoms developed and the lamb was killed 20 days after the start of the experiment, i.e. 17 days after the ticks had been applied.

At post-mortem examination the spleen was found to be enlarged, there was also some enlargement of most of the lymph-glands. There were a number of small white scars on the surface of the liver. Cultural examination of the viscera showed a heavy infection of haemolytic staphylococci in the spleen; a few haemolytic staphylococci were also isolated from the lungs and liver. Cultures from individual liver scars were negative. Positive cultures were obtained from the precrural and supramammary lymph-glands.

Lamb 227.

The/

The skin of the thighs and belly were contaminated with strain 1468 and female ticks from the field were applied. A temperature rise was noted from the 5th to the 9th day. Tick-borne fever was confirmed by examination of blood smears and by passage to a susceptible lamb. On the 8th day, 6 female ticks were found to have attached. A swab from the thighs showed a heavy infection of staphylococci. A batch of nymphs was applied. Unfortunately none of these nymphs attached. No further symptoms developed and the lamb was killed on the 21st day.

No marked abnormality was seen on post-mortem examination. No haemolytic staphylococci were isolated in culture from the viscera, lymph-glands or skin.

On each occasion on which staphylococci were isolated in these experiments, a subculture was sent to Mr. Williams Smith who determined the bacteriophage type. All the strains were type X2. Strains 6591 and 1468, and three of the five strains used on lambs 225 and 226 are also X2.

Discussion. In spite of the numerous failures, these experiments show that a generalised staphylococcal infection can be set up in lambs by feeding ticks on skin which has been contaminated with staphylococci. Bacteriological confirmation of such generalisation was obtained in lamb 226.

Although cultural examination of the abcess in the liver of lamb 200 was negative there is a possibility/

possibility that this case had also passed through a generalised infection and that the infection had been overcome before the lamb was killed one month after the start of the experiment. Abcesses which fail to yield staphylococci on culture are occasionally found in field cases of pyaemia which may yield positive cultures from other parts of the body.

The finding of a single colony of haemolytic staphylococci in culture from the hepatic lymph-gland of lamb 225 may indicate that in this case also generalisation had occurred.

None of these lambs showed typical symptoms of tick pyaemia of which the most common manifestation in field cases is lameness due to abcess formation in the joints. No sign of abcess formation was detected in lamb 226 which was in good condition when killed and had shown no signs of ill-health apart from a temperature rise during the attack of tick-borne fever. As this lamb was killed on the 20th day the further course of the infection must remain in doubt. McDiarmid (1946 a.) records deaths of lambs in the field from the septicaemic phase of the disease. While in the two districts described in Part I such cases were rare, it is possible that a number of lambs pass through a septicaemia from which they recover. In other cases the infection may become localised in an abcess in the viscera, recovery again ensuing. Lamb 200, in which a sterile abcess was present in the liver, may be an example of this type of case.

Although observations in the field have shown that/

that male lambs are more susceptible to tick pyaemia than female lambs, lamb 226, in which a generalised infection was definitely established, was one of three females used in these experiments, the remainder of the subjects being males.

Of the 7 lambs which were examined post-mortem, 5 showed infection of the lymph-glands on the lymph-drainage system of the contaminated areas of skin. This finding is discussed in the following section which deals with preliminary studies of the manner in which the staphylococci invade the lamb's body.

The effect of tick-borne fever on the occurrence of tick pyaemia.

Taylor et al (1941) failed to produce pyaemia by subcutaneous injection of staphylococci in lambs which had been infected with tick-borne fever. The lambs used in this experiment were however four months old which is considerably older than naturally occurring cases.

McEwen (unpublished) has found that, in a group of 46 lambs on tick-infested pasture, 84% developed tick-borne fever during the first six weeks of life. Thus, if this disease is a factor in the development of tick pyaemia, the majority of lambs on tick-infested pastures are exposed to its effects.

Of the lambs which developed tick-borne fever in the experiments under discussion, Nos. 201 and 227 acquired the infection from the ticks used in the experiments; while Nos. 225 and 226 were artificially infected./

infected. A difference in experimental method in lamb 226, in which the staphylococcal infection became generalised, and the others, was that the ticks were applied to the area contaminated with staphylococci during the febrile reaction to tick-borne fever, while in the other lambs the ticks were applied before this reaction had developed.

Under field conditions lambs are exposed to continual reinfestations of ticks. It is possible that cases of tick pyaemia are produced by ticks which attach to skin carrying a staphylococcal infection, during an attack of tick-borne fever set up by an earlier tick infestation. An attempt to reproduce these conditions was made with lamb 227, a batch of nymphs being applied during the febrile reaction to tick-borne fever which had been produced by adults applied previously. Unfortunately none of the nymphs attached. In addition, this lamb, being 6 weeks old at the start of the experiment, may not have been a suitable subject for a transmission experiment.

The case of lamb 200 may indicate that an attack of tick-borne fever is not always necessary for the generalisation of the staphylococcal infection; but the diagnosis of staphylococcal pyaemia in this lamb must remain doubtful as the organism was not recovered at post-mortem examination.

Conclusions. In one out of twelve experiments, a generalised staphylococcal infection was established/

established in a lamb by feeding ticks on skin which had been contaminated with a staphylococcal culture.

Further work is required to determine the factors which are necessary for the production of this condition, and also to establish whether the lamb's resistance to staphylococcal infection is lowered by an attack of tick-borne fever.

Preliminary Studies on the Mode of Entry of the Staphylococcus into the Lamb's Body.

The generalisation of the infection in cases of tick pyaemia indicates that the staphylococci gain access to the blood-stream. The manner by which this invasion occurs, however, remains obscure. The following modes of entry of the infection must be considered:-

(a) The mouth-parts of ticks, feeding on contaminated areas of skin may penetrate cutaneous blood-vessels and set up a septicaemia directly.

A disease very similar to naturally occurring tick pyaemia can be produced by injection into the jugular vein of a suspension of staphylococci. As is detailed in Part III of this thesis, an infective dose of 1 c.c. of a 1/1000 dilution of a No. 8 Brown's scale suspension of strain 1468 was sufficient to produce a pyaemia in lambs up to six weeks old. The period between injection and the first symptoms in these lambs was short; the majority showed signs of lameness within two days. None of the lambs which failed to show symptoms within five days of infection developed the disease. The data given in Part I and the case of lamb 226 recorded in the previous section suggest that in the naturally occurring disease the incubation period is greater than five days.

(b) The tick mouth-parts may carry infection into the skin and set up a local lesion which later liberates staphylococci into the blood stream.

Taylor et al (1941) failed to produce a generalised infection in lambs by the subcutaneous injection of large doses of staphylococci. Local lesions only developed.

The examination of sections of skin, showing the mouth-parts of attached ticks in situ (see following section) suggests that the mouth-parts rarely penetrate into the subcutaneous tissue. The effect of intradermal inoculation of staphylococci was tried.

Intradermal injections of staphylococcal suspensions, in amounts of 4 to 8 times the minimum infective dose by the intracardial route, were given to five guinea-pigs. Local lesions developed in every case but in none did the infection become generalised.

A massive dose of staphylococci was injected intradermally in the back of a rabbit. A large local lesion developed and was followed by sloughing of an area of skin 25 m.m. in diameter. No signs of generalisation of the infection were shown. Complete resolution of the local lesion occurred in six weeks.

A ewe lamb, 18 days old, was given by intradermal injection, an inoculum equal to 900 infective doses by the intravenous route. The inoculum was given at two sites on either side of the neck. Large firm swellings developed at the sites of inoculation. The swellings extended ventrally to meet in the mid-line of the neck. On the 15th day after the injections, abcesses pointed at the sites of inoculation leaving discharging/

discharging sinuses. These sinuses had healed on the 25th day but a firm swelling remained at the mid-line of the neck. This swelling was still present two months later. Apart from slight stiffness on the first day after inoculation, the lamb showed no signs of general infection.

Thus it appears that a generalised infection is not readily set up by either subcutaneous or intradermal injection of large numbers of staphylococci.

(c) There may be a break-down in the barrier to infection normally present in the lymph-glands situated on the lymph drainage system of the tick-infested skin areas.

Henderson (1944) has shown that, in cattle, solutions and particulate substances when injected intracutaneously are conveyed rapidly to the lymph-gland draining the injection site. To confirm that this was also true in sheep the following experiment was carried out:-

An area on the left side of the neck of a ewe was clipped and 0.5 c.c. of a suspension of staphylococci was injected intradermally, 4" in front of the prescapular lymph-gland. The ewe was shot ten minutes later and the prescapular lymph-gland was removed as quickly as possible. A culture made from the gland showed the presence of numerous staphylococci. Numerous staphylococci were also seen in sections of the gland.

That/

That the lymph-glands on the lymph drainage system of areas of infected skin act as a barrier to the spread of infection is confirmed by the fact that in five of the seven lambs which were examined post-mortem during the experiments detailed in the previous section, a lymph-gland infection of this nature was found.

Although the possibility of direct intravenous inoculation of staphylococci by the tick mouth parts exists it seems more probably, on account of the apparent length of the incubation period in field cases, that infection by one or other of the remaining methods of infection is the common one.

As simple intracutaneous or subcutaneous injection of staphylococci failed to set up generalised disease it appeared possible that the secretion of the salivary gland of the tick contained some factor which enabled the staphylococci to invade the blood stream. The following experiments were carried out in an attempt to establish this point:-

Studies on an extract of the salivary glands of Ixodes ricinus.

The only published work on the secretions of Ixodes ricinus is a paper by Sabbatani (1898). Following the work of Haycraft on the anticoagulant in the secretion of leeches, Sabbatani demonstrated the presence of a similar anticoagulant in extracts made from the minced bodies of adult Ixodes ricinus, and concluded that similar ferments were present in all blood-sucking/

blood-sucking animals. The action of the enzyme could be demonstrated in vitro or by intravenous injection. The effect was most marked in the dog, less in the cat, less again in herbivores and least of all in the sheep. The enzyme acted by preventing the action of fibrinogenase.

In the following investigation an attempt was made to isolate the secretion of the salivary glands and study its properties.

Method. Partly engorged female ticks were collected from sheep. Dissection of the salivary glands was carried out under a binocular microscope giving a magnification of x 10, as follows:- The tick was placed in a watch-glass in a drop of normal saline. A blunt needle was pressed against the dorsal surface of the tick just behind the scutum thus pressing the intestinal diverticulae towards the hind end of the body. A longitudinal incision was then made with a sharp needle through the scutum. The pressure of the blunt needle caused the viscera at the front end of the body to extrude through the incision. In most cases the salivary glands were easily visible, as grape-like bunches of clear globules, lying among the excretory tubules and the respiratory tree. Portions of the anterior intestinal diverticulae were usually also extruded. The salivary glands were teased away from the other viscera and transferred to a fresh watch-glass using a capillary pipette.

The salivary glands thus isolated were washed several/

several times in saline and ground up in a small quantity of sand using the bottom of a test-tube. Saline was added to give a volume of 1 c.c. for every 10 sets of salivary glands. The salivary gland suspension was centrifuged to remove the sand and particles of tissue, transferred to a small vaccine bottle and stored in the cold room.

Anticoagulation test:- The following technique was adopted to demonstrate the presence of an anticoagulant in the salivary gland extract. Diminishing volumes of the extract were pipetted into a series of 4 test-tubes of 8 m.m. diameter. Normal saline solution was then added to each tube in the series to bring the volume up to 1 c.c. and an additional tube containing 1 c.c. of saline only was added to the series. A syringe and needle were washed out with saline solution and the needle filled with saline. 10 c.c. of blood was drawn from the jugular vein of a lamb and 2 c.c. was at once added to each of the tubes. The tubes were shaken to mix the blood and the extract, and left at room temperature. Examination for clot formation was carried out by tilting the tubes every minute for the first 20 minutes and thereafter at longer intervals. Clotting time was taken to be the interval from the time of taking the blood-sample until the time when the tube could be inverted without loss of fluid.

Results:- The tick salivary gland extract was found to have a marked anticoagulant action on sheep blood. The results of experiments on two preparations/

preparations of extract are given in the following tables:-

TABLE XXII

Test on extract prepared from ticks collected on 14/3/46.

Tube no.	Volume of extract	Volume of saline.	Volume of blood.	Clotting time
1	0.50 c.c.	0.50 c.c.	2.0 c.c.	No signs of clotting in 24 hours.
2	0.20 c.c.	0.80 c.c.	2.0 c.c.	
3	0.10 c.c.	0.90 c.c.	2.0 c.c.	23 minutes.
4	0.05 c.c.	0.95 c.c.	2.0 c.c.	27 minutes.
5	0.00 c.c.	1.00 c.c.	2.0 c.c.	16 minutes.

TABLE XXIII.

Test on extract prepared from ticks collected on 3/4/46

Tube No.	Volume of extract	Volume of saline.	Volume of blood.	Clotting time.
1	0.50 c.c.	0.50 c.c.	2.0 c.c.	No signs of clotting in 24 hours.
2	0.20 c.c.	0.80 c.c.	2.0 c.c.	
3	0.10 c.c.	0.90 c.c.	2.0 c.c.	
4	0.05 c.c.	0.95 c.c.	2.0 c.c.	
5	0.00 c.c.	1.00 c.c.	2.0 c.c.	20 minutes

These results show that 0.2 c.c. to less than 0.05 c.c. of this extract delays clotting in 2 c.c. of sheep blood for 24 hours. As 1 c.c. of extract was prepared from the salivary glands of 10 ticks the above finding may be stated as follows - extract from the salivary/

salivary gland of from 2 to less than 0.5 ticks delays clotting in 2 c.c. of sheep blood for 24 hours.

Sabbatani, working with an infusion from macerated whole ticks, 1 c.c. of infusion being equivalent to 1 tick, found that 8 c.c. of infusion prevented clotting in 25 c.c. of sheep blood for 24 hours. In other words infusion from 0.64 ticks prevented clotting in 2 c.c. of sheep blood for 24 hours.

Thus preparations of similar anticoagulant potency may be obtained either by using macerated whole ticks or by using dissected salivary glands. It would therefore appear that the anticoagulant factor recorded by Sabbatani from whole ticks, is present in the salivary glands.

In the tubes in the above experiments, in which clotting did not occur, the corpuscles settled to the bottom leaving clear amber coloured plasma. No signs of haemolysis were detected in any of the experiments. It would therefore appear that the salivary gland extract does not contain a haemolysin.

As spreader substances resembling hyaluronidase have been recorded from various animal secretions (Leech extract - Claude 1937. Snake venom - Duran-Reynals 1939), the salivary gland extract from ticks was examined for this property.

Tests for spreader substance:- The hair was removed from the back and flanks of a white rabbit by clipping and depilating with barium sulphide paste. An injection/

injection consisting of 0.1 cc of salivary gland extract and 0.1 c.c. of a 1/5 dilution of India ink was made intracutaneously on the right flank, ½" from the spines of the lumbar vertebrae. A control injection containing 0.1 c.c. of saline and 0.1 c.c. of a 1/5 dilution of India ink was made at a similar site on the left flank.

Twenty minutes after inoculation, the injection site on the right flank was more oedematous than that on the left. The pigment of the India ink was obscured by the oedema. Four hours later there was marked oedema and a patch of bluish discolouration due to the spread of India ink particles on the right flank. The dimensions of the area of spread were 36 m.m.X 22 m.m. the long axis being ventral to the site of inoculation. The corresponding area on the left flank measured 6 m.m. X 4 m.m. There was a small firm swelling and no oedema.

After 24 hours the oedematous area on the right flank extended 40 m.m. X 20 m.m.. The discoloured area was about the same size but the edges were very indistinct. There was a slight oedema round the site of inoculation on the left flank but very little spread of the India ink. Eight days later the oedema and inflammatory reaction had completely subsided. The area of spread of the India ink was much more clearly visible. The measurements of the discoloured areas on the flanks were now - right flank 80 m.m. X 20 m.m., left flank 12 m.m. X 12 m.m..

The wool was clipped from both sides of the neck of a 20 day old lamb. Intracutaneous injections of/

of 0.2 c.c. of salivary gland extract plus India ink and saline plus India ink in the same proportions as in the previous experiment, were made on the right and left sides of the neck respectively. The results of inoculation in this lamb were in every way similar to those in the rabbit. A large oedematous reaction developed on the right side of the neck reaching a maximum size 5 hours after injection. A small firm swelling developed on the left side. Both swellings had subsided on the sixth day leaving areas of skin pigmented by the India ink. The pigmented areas measured:- right side of neck - 60 m.m. X 50 m.m.. Left side of neck 10 m.m. X 10 m.m.. There was no temperature reaction during this period.

Thus the injection of the salivary gland extract from Ixodes ricinus along with India ink produces spread of the ink particles in the surrounding area of skin. The spreading effect produced by hyaluronidase when injected along with India ink has been likened to the diffusion of a drop of ink on blotting paper. The spread of salivary gland extract and India ink differed from this phenomenon in that it was not so rapid and was accompanied by some inflammation and marked oedema.

A sample of the extract was tested by Dr. Hale of the Lister Institute for the presence of hyaluronidase with negative results.

The effect of intracutaneous injection of staphylococci along with salivary gland extract was tested in the following experiments:-

A/

A white guinea-pig was depilated on both sides of the body. A No. 8 Brown's scale suspension of strain 1468 was prepared. 0.1 c.c. of this suspension plus 0.1 c.c. of salivary gland extract was injected intracutaneously into the right flank, and 0.1 c.c. of suspension plus 0.1 c.c. of saline into the left flank.

24 hours after inoculation the site on the right flank was surrounded by a raised and reddened swelling measuring 30 m.m. X 20 m.m. the long axis being ventral to the inoculation. The site on the left flank was surrounded by a raised plaque 10 m.m. X 10 m.m. There was little reddening.

Two days later abcesses had pointed at both sites. The abcess on the right flank was about twice the size of that on the left.

No signs of generalised disease appeared. The guinea-pig was killed seven days after inoculation. The skin containing the lesions was dissected off. Both lesions were surrounded by a reddened area which was most clearly visible from the under surface of the skin. These areas measured - right flank - 20 m.m. X 14 m.m., left flank - 5 m.m. X 5 m.m.

Cultures from both lesions yielded a few α/β haemolytic staphylococci. Cultures from the heart, liver, spleen and kidneys were negative.

The wool was clipped from both sides of the neck of a ram lamb aged 23 days. 0.1 c.c. of a number 8 Brown's scale suspension of strain 1468 plus 0.1 c.c. salivary gland extract was injected intracutaneously into/

into the right side of the neck, and 0.1 c.c. of the suspension plus 0.1 c.c. of normal saline into the left side of the neck. Each of these inocula is equivalent to 300 minimum infective doses by the intravenous route to a lamb of this age.

The following day, 21 hours after inoculation, the lamb was very ill. The skin over the injection sites was stiff and reddened. Extending from the injection site on the right of the neck was a marked oedema which involved the right side of the neck and shoulder, the skin over the lower surface of the thorax, the right-fore leg to below the carpal joint and the left fore-leg to below the elbow. There was little oedema round the injection site on the left side of the neck. Cultures were made from blood samples taken from the marginal vein of the ear and from the caudal veins, but this examination failed to show the presence of a blood infection with staphylococci.

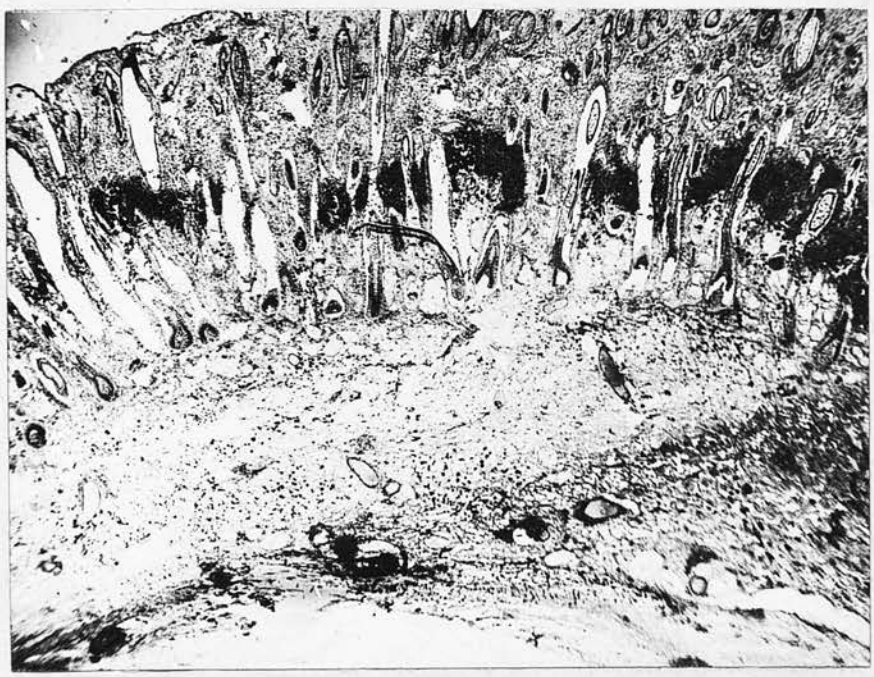
The lamb died 33 hours after inoculation. Post-mortem examination showed that there had been some further extension of the oedema which now involved the right foot, the left leg down to the carpal joint and the anterior two inches of the skin of the abdomen. Cultural examination showed the presence of numerous α/β haemolytic staphylococci in both lesions and in both the prescapular lymph glands. A culture made from the margin of the oedematous area over the abdomen was negative/

Plate 1.

Lesion produced by staphylococci plus salivary gland extract.

X 16.

Plate 2.



Lesion produced by staphylococci plus saline.

X 16.

negative. The muscle on the right side of the neck had a pale bleached appearance. The liver and kidneys were congested and there was congestion of the dorsal margin of the right lung. The heart was flabby and the heart muscle was pale. Cultures from all the viscera were negative.

Although a definite diagnosis was not reached, it appears possible that the death of this lamb was due to shock from the oedema combined with absorbtion of staphylococcal toxins from the local lesions.

Sections were prepared from the lesions at the inoculation sites. These are illustrated in Plates 1 and 2. Examination of these sections shows that, although the lesion on the right side of the neck is about twice the size of that on the left they are otherwise similar in character. There is a central mass of necrotic tissue in the margins of which are very numerous clumps of staphylococci, surrounding this necrotic mass is a dense zone of cellular infiltration. The lesion resulting from the injection of salivary gland extract along with staphylococci showed oedema of the tissue outside the zone of round cell infiltration.

These experiments show that although the injection of salivary gland extract along with staphylococci failed to produce generalised disease, a more severe local reaction was produced than that caused by the injection of staphylococci alone.

A/

A difference between experiments of this nature and the sequence of events occurring in the field is that by experiment a single large dose of staphylococci and salivary gland extract is given, while under natural conditions the flow of salivary secretion into the skin will continue during the period of attachment of each tick. In addition a contaminated skin area on a lamb will be subjected to repeated re-inoculations as fresh unfed ticks become attached to it.

Further experiments could be conducted by giving repeated small injections of staphylococci and of salivary gland extract, but it would be impossible to reproduce the continual infiltration of an area with salivary gland extract which must occur in nature.

Plate 3.

Sagittal section of a nymph attached for less than 24 hours to the skin of a lamb.

X 80.

Preliminary Studies on the Histopathology of Tick-bite.

A histological study of the position of the mouth-parts of the tick and the reaction of the surrounding tissue during tick-bite was made.

Methods. Pieces of skin at the site of tick attachment were fixed in ordinary laboratory fixatives and blocked in paraffin. When the position of the mouth-parts was required, serial sections were cut parallel to the sagital plane of the tick. Owing to the toughness of the chitin comprising the cuticle of the tick, a certain amount of distortion in the sections was unavoidable. This was especially noticeable in the sections which included the mouth-parts of the tick. The sections were stained with haematoxylin and eosin.

Plate 3 shows a sagital section of a nymph attached for less than 24 hours to the skin of a lamb. (In this section the mandibular tube has been distorted and lies across the hypostome instead of along its dorsal surface).

There is little tissue reaction at this early stage of attachment, but an area of coagulative necrosis surrounding the proximal half of the mouth-parts is present. The absence of tissue reaction in this section may indicate that secretion from the salivary glands does not start immediately on attachment.

Plate 4.

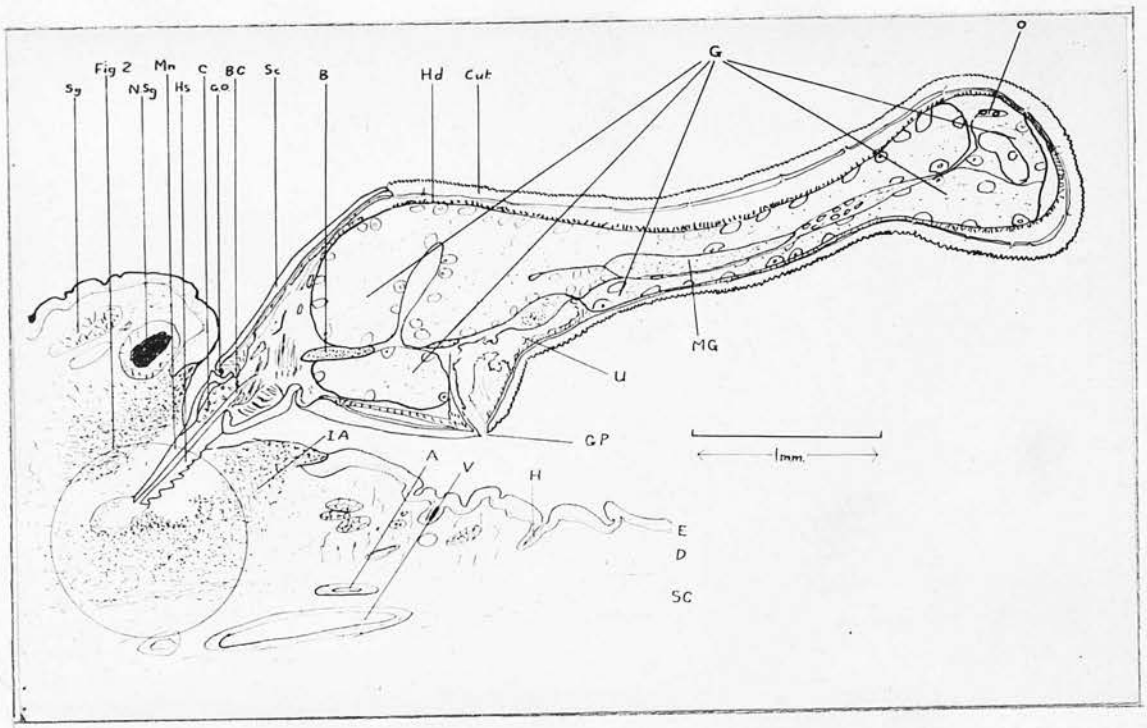


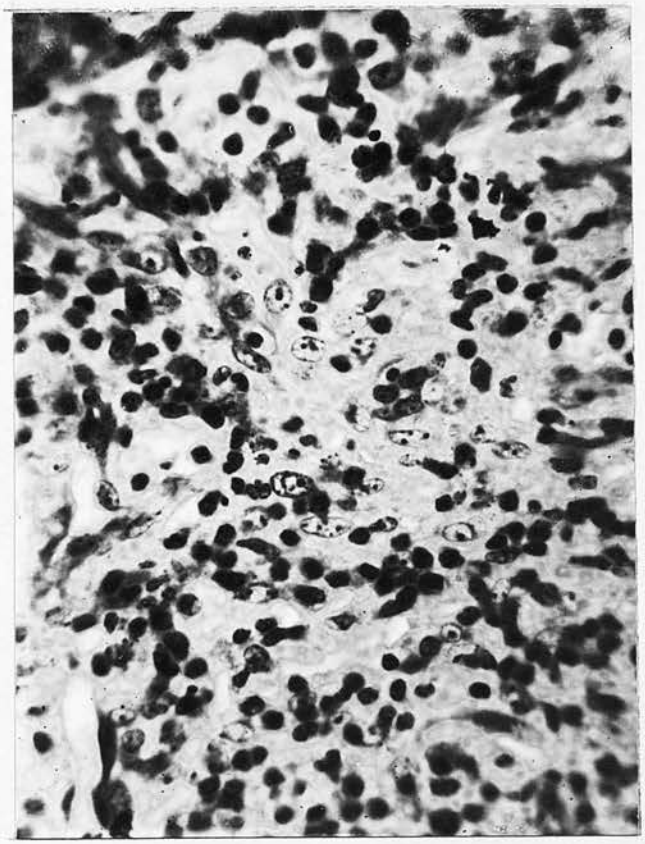
Plate 5.

Plate 4. Drawing of a sagital section of a partly-engorged female tick attached for 5 to 8 days to the skin of a lamb. X 25.

A - arteriole.
B - brain.
D - dermis.
B.C.- buccal cavity.
E - epidermis.
C. - capitulum.
H - hair follicle.
Cut.- cuticle.
I.A.- area of round-cell infiltration.
G. - gut diverticulae.
G.O.- Gene's organ
N.Sg.-necrotic sebaceous gland.
G.P.- genital pore.
S.C.- subcutis.
H.d.- hypoderm.
S.g.- sebaceous gland.
H.s.- hypostome.
V. - venule.
M.G.- mid gut.
Fig.2 - Circle enclosing area shown in Plate 5.
M.n.- mandible.
O. - ovary.
Sc. - scutum.
U. - uterus.

Plate 5. Tissue surrounding the mouth parts of the tick shown in plate 4. X 80.

Plate 6.



Cellular reaction in cutis due to tick-bite.

X 400.

The lesions as seen in Plates 4 and 5 may be described as follows:- Round the axis of the mouth-parts there is a zone of coagulative necrosis. This is surrounded by an area infiltrated by round cells and eosinophils. This zone of coagulative necrosis may serve to fix the mouth parts in the skin. When detaching engorged or partly engorged ticks from sheep in the field, it is frequently noticed that the mouth-parts bring away a ring of necrotic tissue from the skin. At the apex of the mouth-parts is a space containing red blood cells, polymorphs and lymphocytes. This space presumably forms a reservoir from which the tick draws the blood necessary for engorgement. The flow of blood into the space may be helped by the anti-coagulant present in the salivary secretion of the tick.

In the deeper part of the cutis, below the infiltrated area and surrounding its margins, is a tissue reaction characterised by the presence of numerous large epithelioid cells with faintly staining nuclei. Plate 6 is a high power photograph of this tissue.

This reaction may extend for a considerable distance from the site of tick-bite and in some of the sections of skin to which ticks had been attached, it was seen to involve the subcutis.

As was described earlier in Part II, when dealing /

Plate 7.

Tissue from tick-bite infected by staphylococci.

X 80.

dealing with the feeding of ticks on skin contaminated with staphylococci, an infected lesion occurred on the tail of lamb 201. This lesion resulted from the attachment of nine female ticks to an area of skin about ½" in diameter. The lesion consisted of a sinus filled with blood-coloured fluid lying under the area of attachment. Cultures from the contents of the sinus and from the attached ticks yielded growths of staphylococci.

Plate 7 is a photograph of tissue from the margin of this lesion. In it can be seen spaces filled with blood surrounded by granulation tissue. There is a much more widespread infiltration of polymorphs and lymphocytes. In non-infected tick-bites this infiltration is confined to the immediate neighbourhood of the mouth-parts of the tick (see Plates 4 and 5). Epithelioid cells of the type seen in Plate 6 are scarce in the infected lesion which appears to be more necrotic in character.

Discussion. The examination of sections of tick-bites shows that shortly after the tick attachs a zone of coagulative necrosis is formed round the mouth-parts. Later a space filled with blood appears at the apex of the mouth-parts. Surrounding this space is a tissue reaction characterised by the presence of numerous epithelioid cells. There is a varying amount of leucocytic infiltration into the surrounding tissue. This infiltration is more marked in infected tick-bites than /

than in non-infected bites.

The mouth-parts were not seen to penetrate below the cutis into the subcutis in any of the preparations made. There was also no indication of direct involvement of a blood vessel.

Study of these sections suggests that a possible mode of entry of the staphylococcal infection in tick pyaemia is the escape of infection into the blood stream from an infected sinus such as was found in lamb 201. Such escape would be assisted by the anticoagulant secretion of the tick and possibly also by the oedema producing factor.

PART III.

EXPERIMENTS ON THE PROTECTION OF LAMBS AGAINST TICK PYAEMIA BY THE USE OF STAPHYLOCOCCAL TOXOID.

An experiment in the protection of lambs against naturally occurring tick pyaemia in the field was carried out in a tick infested district in Northern Ireland, (Foggie 1943). In this experiment, of 99 immunised lambs, 3 developed pyaemia, and of 91 untreated control lambs, 16 developed pyaemia.

Further experiments were planned to show:-

(a) That a satisfactory immunity against tick pyaemia could be set up in lambs by the injection of staphylococcal toxoid.

(b) That the injection of staphylococcal toxoid in ewes produced a passive immunity in their lambs.

Experiments on the Immunisation of Lambs with Staphylococcal Toxoid.

Methods. The animals used in these experiments were lambs from a flock of cast Black-face ewes bought from a tick-free hill farm and maintained at Moredun Institute.

Both α and α/β staphylococcal toxoids were employed as immunising agents. The α toxoid was Messrs. Burroughs Wellcome's commercial alum precipitated staphylococcal toxoid. The α/β toxoid was an alum precipitated toxoid prepared from strain 1468 by Messrs. Burroughs Wellcome.

The toxoid was given intramuscularly into the /

the thigh, in doses of from 1 to 10 c.c. Apart from transient stiffness immediately after the injection, no ill effects attributable to the toxoid were observed.

The titre of the α and β staphylococcal anti-haemolysin content of the blood of each lamb was tested at intervals throughout the course of the experiment.

These tests were carried out as follows:- Blood samples of from 20 c.c. to 30 c.c. were taken from the jugular vein and collected in McCartney bottles containing 1 to 2 c.c. of liquid paraffin. The liquid paraffin prevented the clot from sticking to the side of the bottle and facilitated the separation of the serum.

The α unitage of the serum was tested against a toxin prepared from Wood strain CN56 and standardised against Burroughs Wellcome antiserum KCP2029.

Dilutions of toxin which were equivalent to 10 International Standard units, 1 I.S. unit and 0.1 of an I.S. unit were found. These dilutions were used as standards to test sera of 10 to 100, 1 to 10 and 0.1 to 1 units per c.c. respectively. In carrying out the tests, a series of varying amounts of sera was added to the test dose of toxin of the expected unit level. The tubes were shaken and allowed to stand for half-an-hour. A suspension of washed sheep red blood corpuscles was then added, to give 2.5% concentration of red corpuscles in each tube. The tubes were incubated at 37°C. in the water-bath for one hour, transferred to the cold-room overnight and read the following morning. The test was adjusted so that,

in /

in a series, the tube showing slight haemolysis was the end point. As staphylococcal toxin is not stable, each set of tests included a test on an antiserum of known unitage.

For the purpose of estimating β unitage, a toxin was prepared from an $\alpha\beta$ strain of staphylococci (1468). The α unitage of this toxin was found by testing it against antiserum KCP2029 using rabbit red blood corpuscles which are not lysed by β toxin. The α factor in the toxin was neutralised by adding a slight excess of α antiserum (KCP2029). The remaining β factor was then standardised against Burroughs Wellcome's antiserum KCP2296 and used to test the unknown sera by the same method as for the α antitoxin.

The resistance of the lambs to staphylococcal infection was tested by injecting a suspension of staphylococci into the jugular vein. An $\alpha\beta$ haemolytic staphylococcus (strain 1468) orininally isolated from the mouth of a ewe (659) and passaged through a guinea-pig was used to set up infection. This strain was seeded into tubes of Worth's medium which were stored at room temperature. To make up the infective dose, five drops of the Worth's medium culture were spread over a Hartley agar slope and incubated for 18 hours. The growth was washed off with normal saline and a suspension equal in density to tube No. 8 on Brown's scale was made up. The infective dose was 1 c.c. of a dilution of this suspension.

Results. In 1945, fifty-five lambs were available for experiment. Sixteen of these lambs were used to find /

a titre of 17 units, (this rose to 40 units following the injection of toxoid); one lamb in the 71 to 87 day group had a titre of 4 units.

All the lambs were bled and their sera were tested at from 3 to 7 days after the last injection of toxoid.

The results of these experiments are summarised in the following table:-

TABLE XXIV.

Administration of toxoid to lambs of different ages.

Age of lambs at injections of toxoid.	Number of lambs in group.	Number of lambs with final titres of	
		1 to 9 units	>9 units.
10 to 14 days	6	0	0
14 to 19 days	4	0	0
20 to 24 days	4	0	0
25 to 28 days	7	4	0
27 to 30 days	7	2	2
30 to 33 days	4	2	0
38 to 48 days	4	0	4
71 to 87 days	3	1	2

These results appeared to indicate that a response to toxoid could be obtained in some lambs at between 25 and 30 days old, but that some lambs would not respond until they were about 40 days old.

As a longer interval between the injections of toxoid might give a better immunological response, the following experiment was carried out in 1946:-

Thirty lambs were used in the experiment. Ten of these lambs were left untreated as controls.

Ten /

Ten lambs were given injections of 2.5 c.c. α toxoid when they were 25 to 27 days old and 5 c.c. of α toxoid when they were 34 to 36 days old. The remaining ten lambs were treated in the same way except that α/β toxoid was used. Blood samples were taken from all the lambs at 25 to 27 days, 34 to 36 days and 40 to 42 days old. These samples were tested for their α and β anti-haemolysin content with the following results:-

TABLE XXV.

Numbers of lambs with raised α anti-haemolytic titres.

Group	Age in days.	Number of lambs with titres of 1 to 9 α units.	Number of lambs with titres above 9 α units.
10 untreated lambs.	25 to 27	2	0
	34 to 36	3	0
	40 to 42	2	1
10 lambs receiving α toxoid.	25 to 27	1	0
	34 to 36	0	1
	40 to 42	1	1
10 lambs receiving α/β toxoid.	25 to 27	1	0
	34 to 36	1	0
	40 to 42	0	1

With one exception the β anti-haemolytic titres of the samples from all the lambs on all occasions were less than 1 unit. The exception was in a sample from a lamb in the α/β toxoid group, on the 42nd day, when a titre of 1.4 β units was found.

Thus, in this experiment, the injection of staphylococcal /

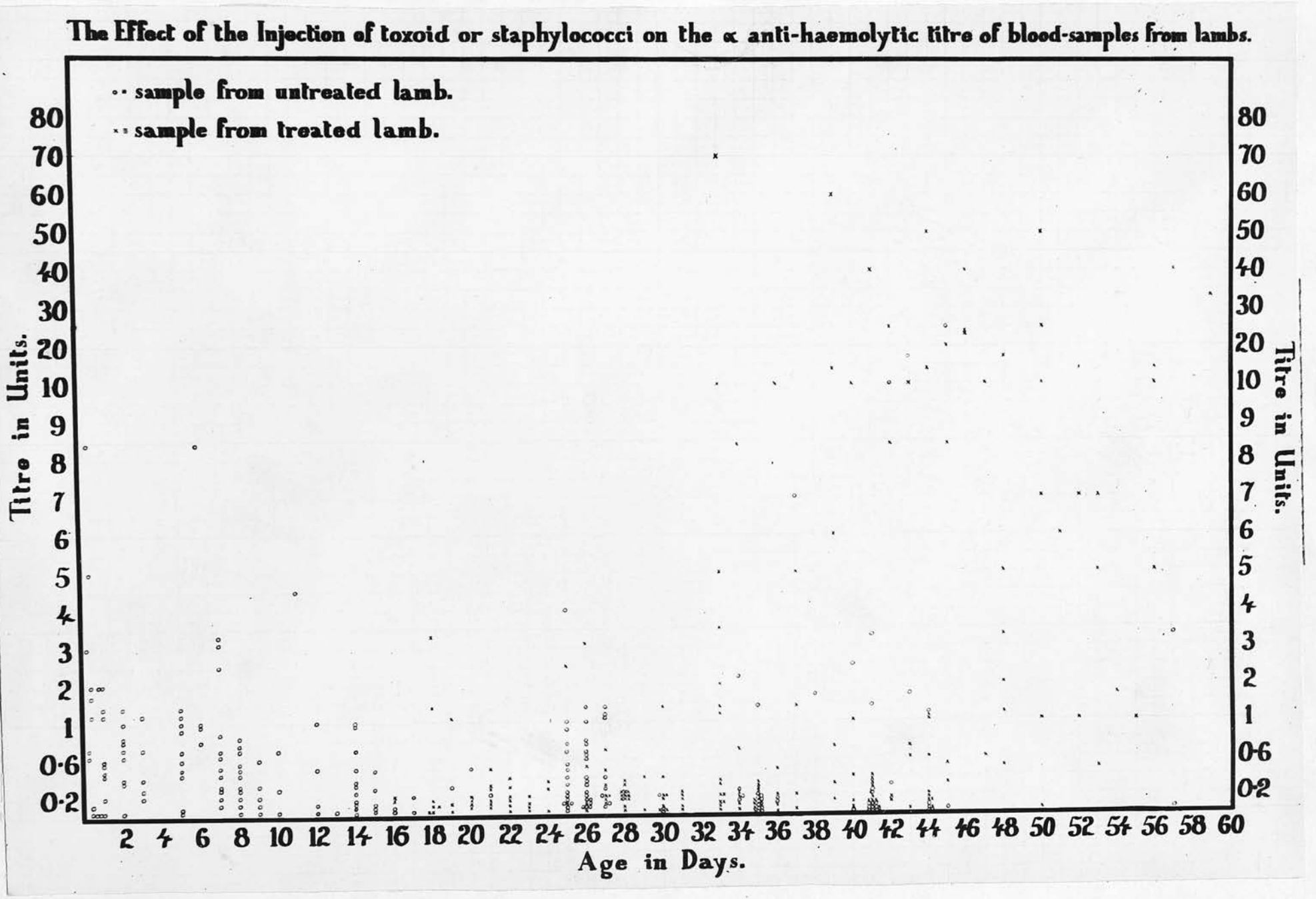

The Effect of the Injection of toxoid or staphylococci on the α anti-haemolytic titre of blood-samples from lambs.
·· sample from untreated lamb.
×× sample from treated lamb.
Titre in Units.
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0·6
0·2
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
Age in Days.

Figure 2.

staphylococcal toxoid did not increase the number of lambs with raised anti-haemolytic titres when compared with the number of untreated lambs with raised titres.

This result is at variance with the results of the experiments conducted the previous year. It is difficult to explain this difference. The nutritional level of the lambs may have some influence on the formation of antibodies as the lambs in 1945 showed a much better thrive than those in 1946.

Although the above experiments were inconclusive, a study of the results of the considerable number of blood-tests done during the course of the experiments indicates the general trend of staphylococcal anti-haemolysin production in young lambs.

In Figure 2, the α anti-haemolytic titre of each blood-sample tested is plotted against the age at which the sample was taken. The figure includes all the samples taken from the lambs in the above experiments, up to the age of 60 days; as well as all samples from lambs used to determine the minimum infective doses as is described later.

In the figure 'o' indicates that the sample was taken from a lamb before it had been given antigen; 'x' indicates that the sample was taken from a lamb which had received antigen, either in the form of toxoid or as an intravenous injection of staphylococci.

In order to simplify the interpretation of

Figure /

Figures 3 and 4.

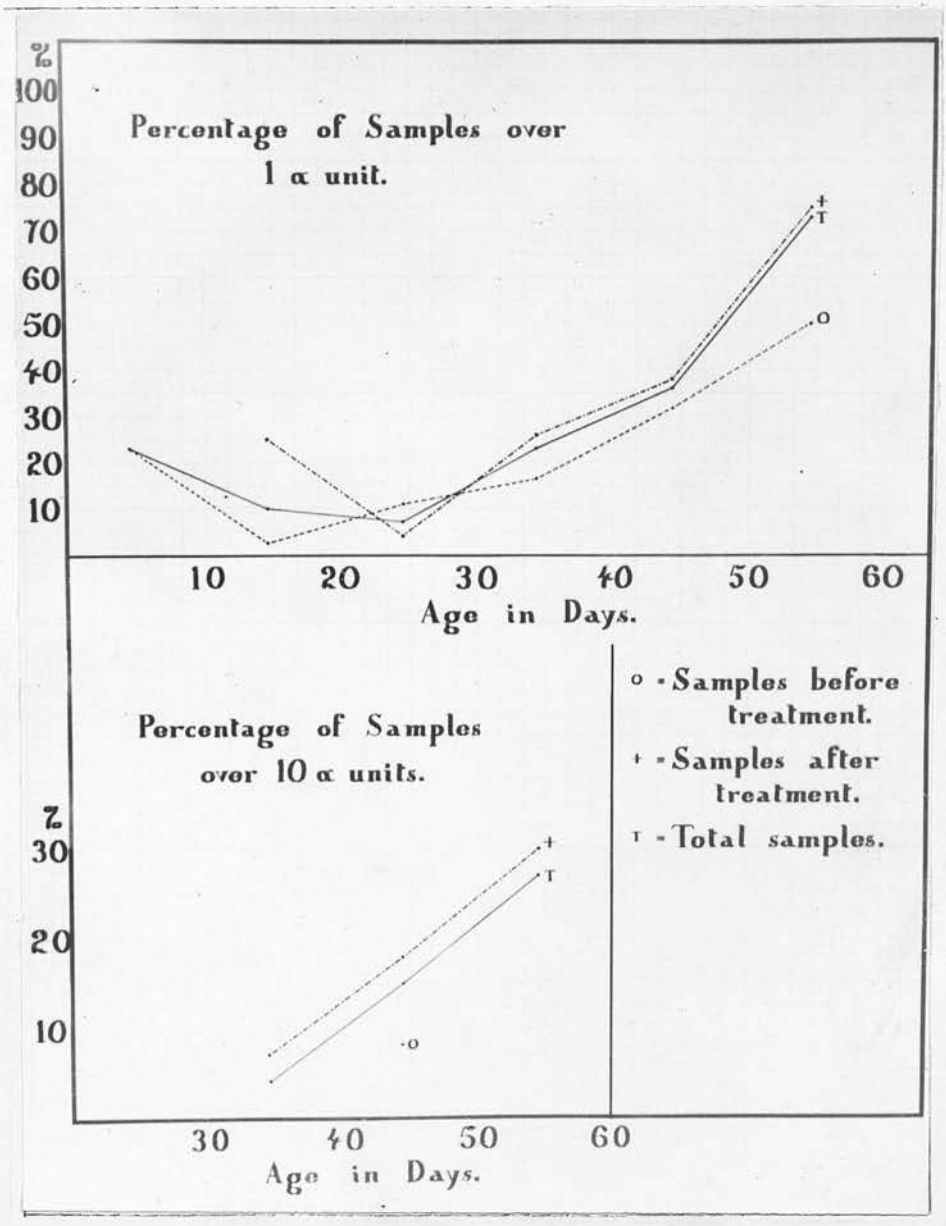


Figure 2, the samples were divided into age groups of 0 to 9 days, 10 to 19 days, 20 to 29 days, and so on. The percentage of the total number of samples which were over one unit, and also the percentages of samples from untreated, and samples from treated lambs over one unit were calculated. These percentages were plotted against the age group of the samples. These curves are shown in Figure 3. This figure shows that in the under-ten age group, twenty-three per cent of the samples were over 1 unit. This percentage drops to 7 in the 20 to 29 day age group and thereafter rises steadily. There is little difference from this general trend in samples from treated and untreated lambs. After the 30th day, the percentage of samples over 1 unit from treated lambs tends to be higher, and that from untreated lambs lower than the percentage of the total samples. The accuracy of the percentages given for treated lambs at 10 to 19 days, and for untreated lambs at 50 to 59 days, is questionable, as these figures were calculated from small numbers of samples.

Figures 2 and 3 show that sera from lambs up to the age of 10 days have appreciable anti-haemolytic titres. This is due to antibodies received from the ewes via the colostrum:- Four lambs were bled before they had sucked their mothers. The titres of all four sera were less than 0.1 of a unit. The lambs were again bled within seven days after they had sucked.

The /

The titres of the sera were now 0.7, 1.2, 1.4 and 1.0 units.

The drop in the curve (Fig. 2) is presumably due to the elimination of these colostral antibodies. A possible explanation of the failure of young lambs to react to injections of staphylococcal toxoid may be that an active immunity can not be established while traces of this passive immunity are present.

In Figure 4 the percentages of the total number of samples, and of the samples from treated lambs, with titres over 10 units are plotted against the age groups. This figure shows that the numbers of samples with titres of over 10 units increased from the 30th day onwards. The percentage of the samples from treated lambs over 10 units is greater than that of the total number of samples. Eight per cent of the samples from untreated lambs were over 10 units between 40 and 49 days. This point is also indicated in the figure.

It would appear from study of Figures 2 and 4 that the injection of antigen was responsible for the production of the majority of the samples with titres of over 10 units.

In order to test the resistance to staphylococcal infection of lambs which had received toxoid, a number of the immunised lambs were infected by intravenous injection of a staphylococcal suspension.

Minimum infective doses for untreated lambs of 17 days old, and for untreated lambs about 40 days old were /

were determined. This was done by inoculating 1 c.c. of varying dilutions of a No. 8 Brown's scale suspension of strain 1468 into the jugular veins of groups of lambs of these ages. The minimum infective dose was taken to be the dilution which produced symptoms of pyaemia within five days of inoculation, the symptoms persisting for at least seven days.

The results of these determinations are given in the following tables:-

TABLE XXVI.

Minimum infective dose for lambs of 17 days old.

Dilution of No. 8 B.S. suspension of strain 1468.	Number of lambs inoculated.	Number of lambs developing pyaemia.
1/100	1	1
1/300	2	2
1/1,000	2	2
1/3,000	5	3
1/10,000	3	2
1/100,000	3	0

The minimum infective dose of lambs of about 17 days old was taken to be 1 c.c. of a 1/3,000 dilution of a No. 8 Brown's scale suspension of strain 1468.

TABLE XXVII. /

Minimum infective dose for lambs of 40 to 42 days old. /

TABLE XXVII.

Minimum infective dose for lambs of 40 to 42 days old.

Dilution of No. 8 B.S. suspension of strain 1468.	Number of lambs inoculated.	Number of lambs developing pyaemia.
1/200	1	1
1/500	1	0
1/1,000	7	6
1/2,000	1	1
1/5,000	1	0

The minimum infective dose for lambs of about 40 days old was taken to be 1 c.c. of a 1/1,000 dilution of a No. 8 Brown's scale suspension of strain 1468.

Except for one lamb in the 17 day age group, which was given the 1/300 dilution and which developed pyaemia, the α titres of all the lambs in these groups were below 1 unit at the time of infection.

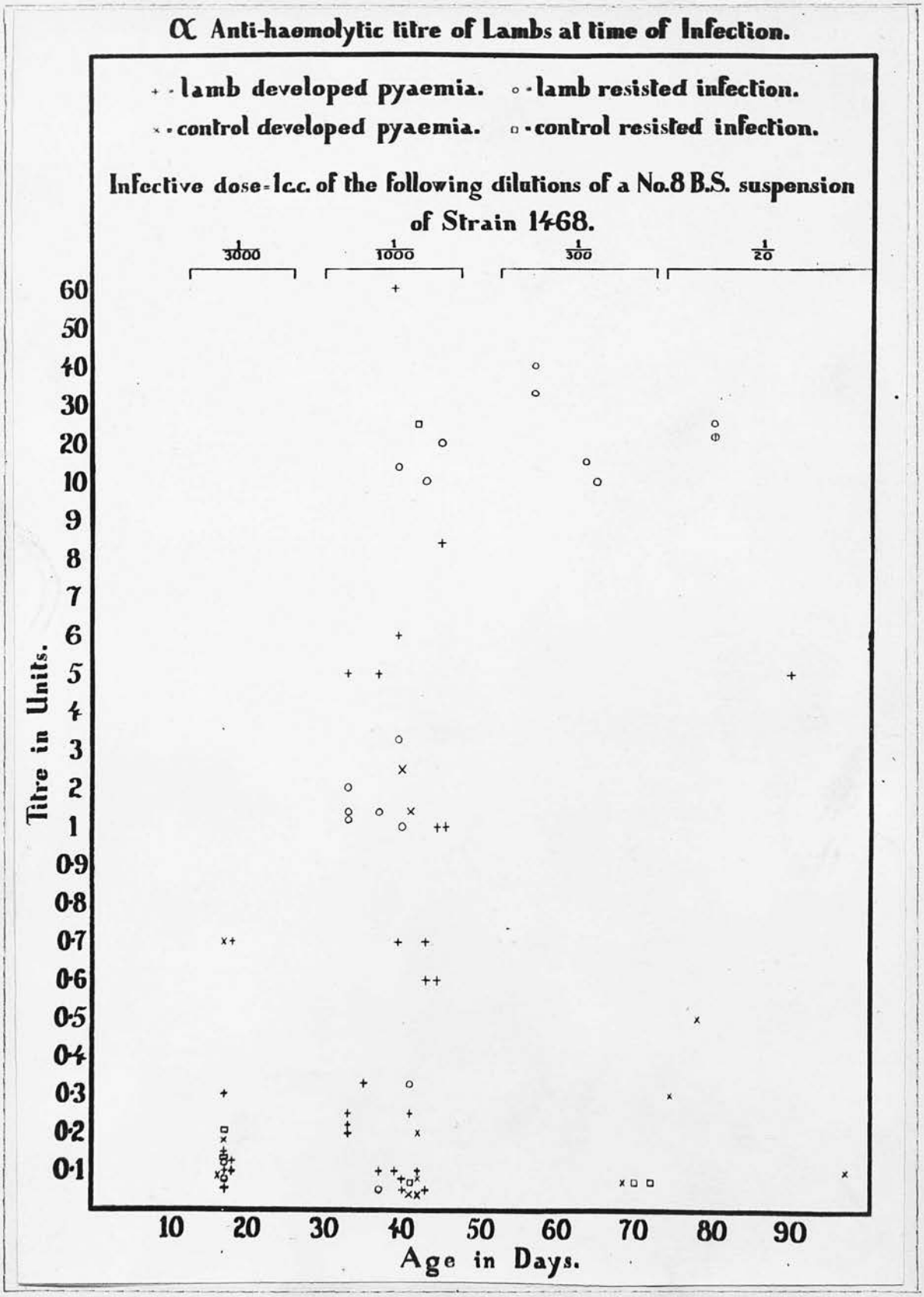
Sufficient lambs were not available to fix minimum infective doses for the older groups of immunised lambs.

Four immunised lambs of 57 to 66 days old were infected with 1 c.c. of the 1/300 dilution. Three untreated control lambs, aged 69, 70 and 71 days, with titres of less than 1 unit, were infected with this dose. Only one of these lambs developed pyaemia. Thus the infective dose for this age group may have been too light.

Six lambs of from 75 to 95 days old were infected with 1 c.c. of a 1/20 dilution. Three of these lambs were untreated controls with titres of

less /

Figure 5.


∝ Anti-haemolytic titre of Lambs at time of Infection.
+ - lamb developed pyaemia.
○ - lamb resisted infection.
× - control developed pyaemia.
□ - control resisted infection.
Infective dose = 1 c.c. of the following dilutions of a No.8 B.S. suspension of Strain 1468.
1/3000
1/1000
1/300
1/20
Titre in Units.
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
10
20
30
40
50
60
70
80
90
Age in Days.

less than 1 unit. The three controls all developed pyaemia; thus this infective dose may have been too heavy.

The results of infecting lambs which had been given injections of toxoid are shown in Figure 5. In this figure the α anti-haemolytic titre of each lamb at time of infection is plotted against the age of the lamb. The result of infection is indicated by the symbol on the figure. One lamb in the 75 to 95 day group showed slight lameness for two days. This is indicated '⊘' in the figure. The titres of untreated control lambs which were infected are also included in the figure.

As is shown in the figure, of 42 lambs with titres of less than 0.9 of a unit, 33 developed pyaemia and 9 resisted infection; of 15 lambs with titres of 1 to 9 units, 9 developed pyaemia and 6 resisted infection; and of 11 lambs with titres of over 9 units, 1 developed pyaemia, 9 resisted infection and 1 showed transient symptoms.

A more valid result may be obtained by confining attention to the titres of the lambs which were infected when they were from 33 to 45 days old. All the lambs in this group received the same infective dose, namely 1/1,000 of a No. 8 Brown's scale suspension of strain 1468.

TABLE XXVIII. /

TABLE XXVIII.

Infection of lambs at 33 to 45 days old.

Titre in units.	Number of lambs which developed pyaemia.			Number of lambs which resisted infection.		
	Immunised	Control	Total	Immunised	Control	Total.
< 0.9	15	4	19	2	1	3
1 - 9	6	2	8	6	0	6
> 9	1	0	1	3	1	4
Total	22	6	28	11	2	13

Thus it would appear that lambs with α anti-haemolytic titres of less than 0.9 of a unit are more susceptible to staphylococcal infection than lambs with titres of more than 1 unit.

That the immunity conferred by a raised titre is not absolute is shown by the fact that four lambs with titres of from 5 to 10 units and one lamb with a titre of 60 units developed pyaemia when infected.

Another factor which must be considered when interpreting the results of these experiments is the individual variations in susceptibility of lambs to infection by staphylococci. During the course of these experiments, 45 lambs with titres of less than 1 unit were inoculated intravenously with the approximate minimum infective dose for their different age groups. Eleven of these lambs failed to develop pyaemia. In the group of lambs used to determine the minimum infective dose for the 33 to 45 day age group, one lamb resisted infection with an inoculum which was twice the minimum infective dose for that group.

Similar /

Similar variations in susceptibility of rabbits to staphylococcal infection are described by Julianelle (1944).

Discussion.

These experiments show that a reaction, by the production of anti-haemolysins, to the injection of staphylococcal toxoid does not occur in lambs below the age of 25 days. Only a proportion of the lambs inoculated between 25 and 45 days old react to toxoid.

On testing by artificial infection, a smaller proportion of the reacting lambs than the non-reacting lambs developed pyaemia, but the presence of a raised titre did not necessarily indicate immunity.

As is already shown in Part I of this thesis, the age incidence of tick pyaemia on Scottish Hill sheep farms is from one to seven weeks, with a maximum incidence of from three to five weeks. The incubation period of the natural disease is probably greater than one week (see Part I). Thus the majority of cases on these farms are infected before they are capable of reacting to toxoid.

In the experiment already quoted (Foggie 1943) an apparently significant degree of protection from tick pyaemia in the field was obtained by the injection of lambs with staphylococcal toxoid.

As there appears to be a discrepancy between the results of the experiments under discussion and the result of the 1943 experiment, a further examination of the conditions of the latter is made.

The /

The management of the flocks on the Northern Ireland farms in the 1943 experiment differs from that most commonly practised on Scottish hill farms in that the ewes are lambed on low tick-free pastures and the lambs are not exposed to tick infestation until they are from two to four weeks old. During the experiment this period was extended until two injections of toxoid had been given, the lambs being then four to six weeks old.

Thus these lambs receivedan injection of toxoid when they were over four weeks old, and may have been capable of reacting.

In order to facilitate identification in the 1943 experiment, all ewe lambs were inoculated with toxoid, the ram lambs being left as untreated controls. The investigations described in Part I showed that, in the farms where the incidence was studied, a significantly greater number of ram lambs than ewe lambs became affected with tick pyaemia. This finding reduces the validity of the results of the earlier experiment.

The method of infection may also have a bearing on the results of the two sets of experiments. A lamb with a moderately high titre may be able to withstand repeated small infections of staphylococci due to tick-bite, although it develops pyaemia when the infection is administered as a single inoculum.

Experiments on the Protection of Lambs against Tick Pyaemia by Immunisation of Ewes with Staphylococcal Toxoid.

An attempt was made to immunise ewes with staphylococcal /

staphylococcal toxoid to see whether sufficient passive immunity was given to their lambs to protect them against artificial infection with staphylococci.

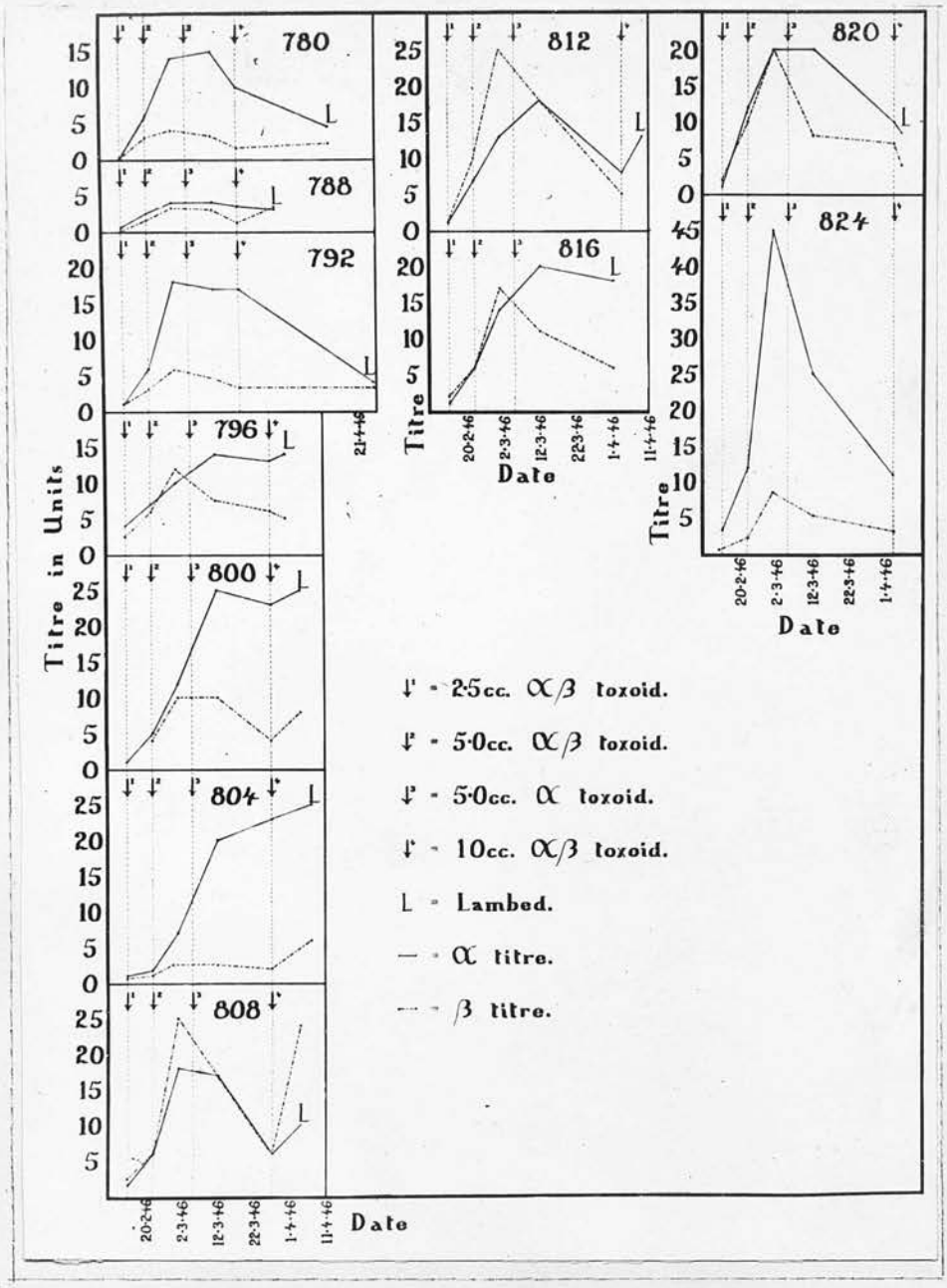
Method.

Eleven ewes were each given three injections of 2.5 c.c. $\alpha\beta$ 5 c.c. $\alpha\beta$ and 5 c.c. α toxoid at intervals of one week from the 15th of February to the 1st of March. A fourth injection of 10 c.c. $\alpha\beta$ toxoid was given as near to lambing time as possible. The ewes were divided into three groups. The first group (Nos. 780, 788 and 792) was due to lamb during the last week of March and received the fourth injection of toxoid on the 19th of March. The second group (Nos. 796, 800, 804 and 808) was due to lamb during the first week in April and received the fourth injection on the 27th of March. The third group (Nos. 812, 816, 820 and 824) was due to lamb during the second week in April and received the fourth injection of toxoid on the 3rd of April. Unfortunately, the dates at which lambing was expected proved to be inaccurate in a number of cases. Thus ewes Nos. 780 and 792 did not lamb for more than three weeks after receiving the fourth dose of toxoid, and ewe No. 816 lambed before the fourth dose had been given. Ewe No. 824 proved to be not in lamb.

The sera from blood samples, taken at each injection and at lambing were tested for the presence of α and β anti-haemolysins.

Thirteen /

Figure 6.



The anti-haemolytic reactions of ewes to injections of staphylococcal toxoid.

Thirteen lambs were born from ten of the immunised ewes. Ewe 812 had one blind teat and a poor yield of milk from the other. Her lamb died three days after birth, before a blood sample had been taken.

Sera from the remaining 12 lambs was tested for α and β anti-haemolysins during the first week of life and at intervals thereafter.

Seven of these lambs were given an infective dose of 1 c.c. of a 1/3,000 dilution of a No. 8 Brown's scale suspension of strain 1468, when they were from 12 to 18 days old.

Results.

The changes in anti-haemolytic titre in the ewes are shown graphically in Figure 6.

In general the α titre rose to a maximum about the 12th of March and then declined. The fourth injection of 10 c.c. $\alpha\beta$ toxoid had only a small effect in raising the α titre prior to lambing. The third injection contained only α toxoid and the peak of the β curve occurred on the 1st of March. There was, in most cases, a more marked increase of β anti-haemolysin following the fourth injection. The mean α and β titres of these ewes at lambing, compared with the titres at lambing of untreated ewes in the same flock, are given in the following table :-

TABLE XXIX.. /

TABLE XXIX.

Mean titres of immunised and untreated ewes.

Description of ewes.	Number of ewes.	Mean α titre in units.	Standard deviation in units.	Mean β titre in units.	Standard deviation in units.
Immunised	11	12.09	±8.167	6.78	±6.708
Untreated	38	2.13	±2.482	2.76	±3.522

There is a highly significant difference between the mean α titres ($t = 6.360$. $P = < 0.001$), and a significant difference between the mean β titres ($t = 2.533$. P. = 0.02 to 0.01). Thus the injection of staphylococcal toxoid increases the antibody content of ewes' sera.

The means of the anti-haemolytic titres of the colostra of eight of the immunised ewes were:- α - 24.2 units; ±13.93 units: β 6.14 units, ±3.49 units.

The anti-haemolytic titres of the lambs from these two groups of ewes are compared in the same way in the following table:-

TABLE XXX. /

TABLE XXX.

Mean titres of lambs from immunised and untreated ewes.

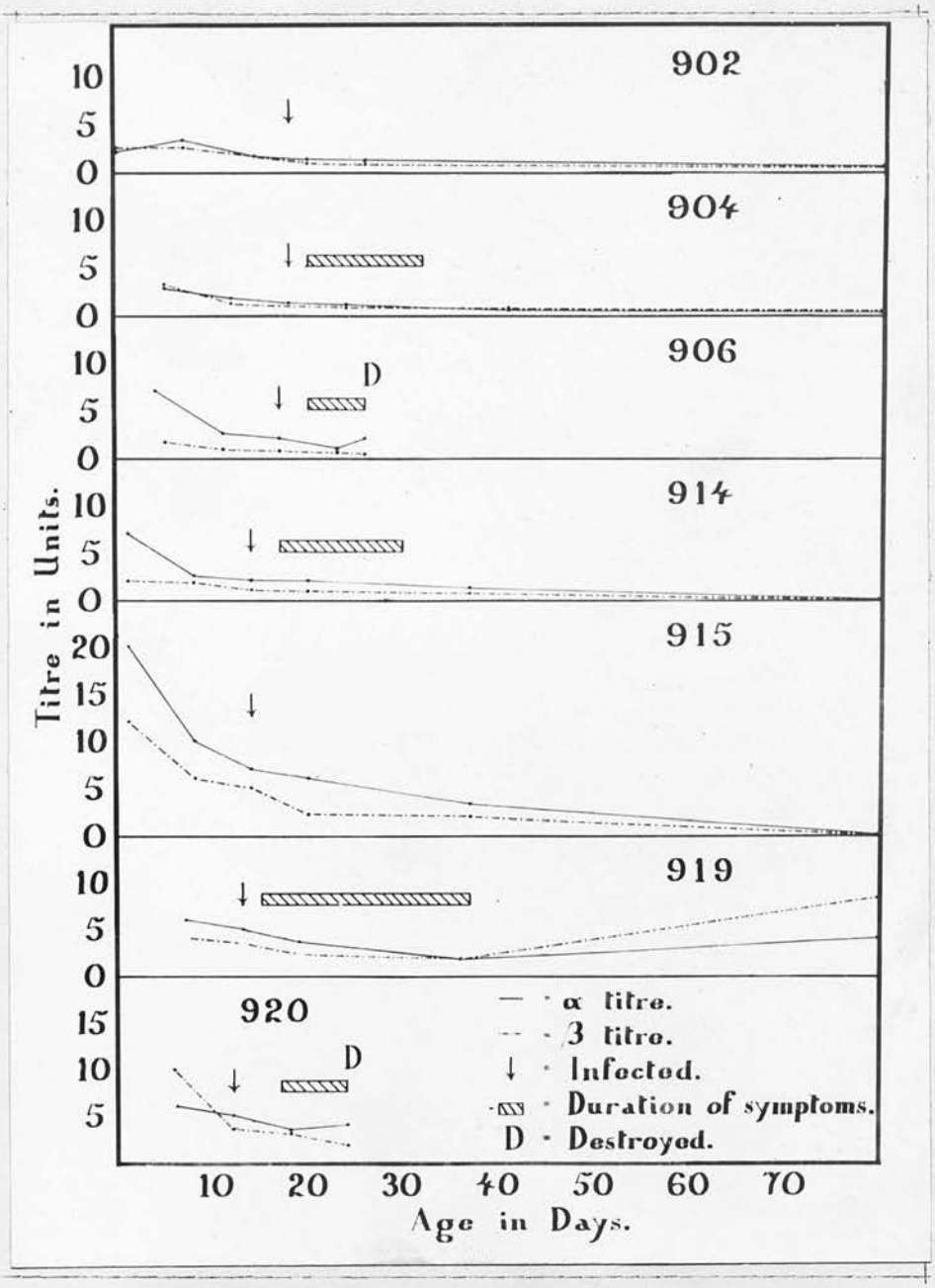
Description of lambs.	Number of lambs.	Mean α titre in units.	Standard deviation in units.	Mean β titre in units.	Standard deviation in units.
1 to 7 days old. Ewes immunised.	12	5.24	±5.459	4.23	±4.061.
1 to 7 days old. Ewes untreated.	48	1.11	±1.393	1.79	±2.929
12 to 18 days old. Ewes immunised.	7	3.41	±2.066	2.29	±1.623
12 to 18 days old. Ewes untreated.	43	0.36	±0.518		

Statistical analysis of these figures shows that, at the 1 to 7 day age level, there is a highly significant difference between the α titres of the two groups of lambs ($t = 4.726$. $P = <0.001$), and a significant difference between the β titres ($t = 2.278$. $P =$ 0.05 to 0.02). There is also a highly significant difference between the α titres of the two groups at the 12 to 18 day level. As the β anti-haemolytic titre was not tested below 1 unit, a mean can not be given for the β titres of lambs from untreated ewes in the 12 to 18 day age group.

Seven of the lambs from the immunised ewes were given an infective dose of 1 c.c. of a 1/3,000 dilution of a No. 8 Brown's scale suspension of strain

1468 /

Figure 7.



The changes in anti-haemolytic titre in lambs from immunised ewes and the results of artificial infection with staphylococci.

1468, when they were 12 to 18 days old. Five lambs developed pyaemia.

The changes in the anti-haemolytic titres and the duration of the symptoms are shown graphically in Figure 7.

This figure shows that there was a steady decline in both α and β titres from the first week of life onwards.

Of the two lambs which resisted infection, one (No. 915) had titres of 7.0α and 5.0β units, and the other (No. 902) had titres of 1.7α and 1.7β units. The titre of lamb 915 was the highest obtained at the time of infection.

The infective dose did not appear to influence the gradual fall in titre in four out of the five surviving lambs, which all had titres of $<1\alpha$ and $<1\beta$ units when they were 10 to 12 weeks old. In lamb 919, symptoms of pyaemia persisted until the 38th day of life. The titre of this lamb was considerably raised by the 12th week of life.

Although the results of this experiment gave little indication that the passive immunity conferred on the lambs from immunised mothers was sufficient to protect the lambs against artificially produced pyaemia, a field experiment was planned to test the effect of such an immunity against the naturally occurring disease.

Field experiment.

A /

Field experiment.

A flock of 368 ewes on The Knowe, Kirkconnel, was used for this experiment. 182 ewes were given two injections of 5 c.c. of Messrs. Burroughs Wellcome's α staphylococcal toxoid prior to the start of lambing; the remaining 186 ewes were left untreated as controls. The first injections were made on the 1st of April and the second injections on the 10th of April. Lambing started on the 14th of April. As the two groups of ewes grazed on the same pastures, the immunised ewes were identified by marking them with keil.

Twenty ewes in each group were ear-tagged. Blood samples were taken from the ear-tagged ewes in the immunised group at each injection and on the 7th of May. All but two of these ewes had lambed by this date. Blood samples were taken from the ear-tagged ewes in the control group on the 1st of April and on the 7th of May, when they had all lambed. The sera from these blood samples were tested for their α anti-haemolytic titres.

The mother of each lamb, which developed tick pyaemia, was identified and a note was made of the group to which she belonged. A final count of the lambs which had contracted tick pyaemia was made at ear-marking time on the 12th of June.

Results.

The results of the examinations of the blood samples from the two groups of ewes are summarised in the following table:-

TABLE XXXI. /

TABLE XXXI.

Mean titres of α anti-haemolysins in sera from 20 immunised and 20 control ewes.

Date.	Immunised ewes.		Control ewes.	
	Mean titre.	Standard deviation.	Mean titre.	Standard deviation.
1/4/46	3.4	±2.82	1.8	±1.59.
10/4/46	51.0	±35.74		
7/5/46	13.8	±7.29	2.1	±2.54

This table shows that the response to the first injection of toxoid was good (2 of the immunised ewes had titres of 140 units at the second bleeding). Four weeks after the second injection, towards the end of the lambing period, the titres had fallen considerably, but they were still significantly higher than those of the control ewes. There was no significant alteration in the titres of the control ewes during the course of the experiment.

The results of the counts of lambs which developed tick pyaemia, in the two groups, are as follows:-

TABLE XXXII.

	Lambs from immunised ewes.	Lambs from control ewes.	Totals.
Normal lambs.	175	182	357
Pyaemia cases.	6	4	10
Totals	181	186	367

$\chi^2 = 0.4692$ $P = 0.5$

Therefore in this experiment there was no significant /

significant difference in the number of lambs which contracted tick pyaemia in the two groups.

Discussion.

The results of these experiments show that, although ewes react to the injection of staphylococcal toxoid by a considerable increase in the anti-haemolytic content of their sera, sufficient immunity is not passed to their lambs to protect them against either artificial or natural infections of staphylococci.

Two factors which may account for this failure are:-

1. The rapid drop in antibody content of the ewes' sera following the rise produced by the injection of toxoid. In order to obtain the maximum degree of passive immunity in the lamb, the ewe would have to be given an injection of toxoid within 8 or 10 days of lambing. This is not practical under hill sheep farming conditions.

2. The rapid elimination of colostral antibodies from the lamb. As is shown in Figure 7, the anti-body content of the sera from most of the lambs from immune ewes drops to a low level within 15 days of birth. Tick pyaemia is a disease of lambs of from 3 to 5 weeks old and not of the newly born lamb. It may therefore be assumed that maternal immunity, which may protect the lamb early in life, has ceased to be effective when the lamb approaches the age of greatest incidence.

Conclusions. /

Conclusions.

It is concluded from the experiments detailed in Part III, that staphylococcal toxoid, administered either to the lambs or to the ewes prior to lambing, is of no value in preventing the occurrence of tick pyaemia.

A possible exception to this finding may be the use of staphylococcal toxoid, by injection into the lambs, on farms in which lambing is conducted on tick-free pastures, the lambs being later exposed to tick infestation. Further experiments are necessary to prove this point.

SUMMARY.

PART I.

The relationship between tick infestation and the incidence of tick pyaemia in two tick-infested districts is discussed. The evidence obtained tends to confirm the opinion that tick-bite is a causal factor in the production of this disease.

PART II.

1. The results of experiments with infected ticks showed that the tick is unlikely to act as a true vector of the causal staphylococcus in tick pyaemia. In ticks infected by feeding on an animal suffering from a staphylococcal septicaemia, the infection did not survive the moulting period.

2. Haemolytic staphylococci were found to be of common occurrence in the natural orifices of ewes and on the skins of lambs.

3. The staphylococci from the above sites were shown to have the same characteristics as the staphylococci from tick pyaemia cases.

4. A staphylococcal septicaemia was produced in one lamb by feeding ticks on skin contaminated with staphylococcal culture. The possible influence of tick-borne fever on the development of tick pyaemia is discussed.

5./

5. An examination of the manner in which the staphylococcal infection becomes generalised is made. An investigation of the properties of tick salivary gland secretion is recorded.

6. A preliminary study of the pathology of tick-bite is made.

PART III.

Staphylococcal toxoid administered either to the lamb or to the ewe before lambing was shown to be of no value in the prevention of tick pyaemia on Scottish hill sheep farms.

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